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Title: ***Schiff's Diseases of the Liver, 10th Edition***

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Editors: **Schiff, Eugene R.; Sorrell, Michael F.; Maddrey, Willis C.**
Title: ***Schiff's Diseases of the Liver, 10th Edition***

Dedication

We dedicate this tenth edition of Diseases of the Liver *to Telfer B. Reynolds, who was a great teacher, mentor, clinician and contributor to the science and practice of hepatology. Furthermore, we dedicate this edition to our wives Dana, Shirley and Ann for their continuing support of our endeavors.*

Editors: **Schiff, Eugene R.; Sorrell, Michael F.; Maddrey, Willis C.**
Title: ***Schiff's Diseases of the Liver, 10th Edition***

Preface to the First Edition

In the recent words of Himsworth, the present time seems to be particularly opportune for reviewing our knowledge of liver disease. A partial list of reasons would include the advances made in the fundamental sciences as they pertain to liver structure and function; the advances in the experimental approach to liver disease; the increased knowledge in the field of viral hepatitis; the newer clinical criteria and concept of hepatic coma, with attention focused on disturbance in the metabolism of ammonia; a better understanding of the pathogenesis and the treatment of cirrhosis; a clearer concept of the metabolic defect in hemochromatosis and the apparent effectiveness of depleting iron stores in the treatment of this disorder; the implication of disturbed copper metabolism in hepatolenticular degeneration; the increasing experience with needle biopsy of the liver; and the surgical attack on portal hypertension.

This book is not intended to be encyclopedic in nature but rather the expression of present-day information pertaining to various aspects of liver disease by a group of authors particularly qualified by their experience, interest, and scientific contributions. The reader may discover certain omissions, but he usually will find these to be matters of lesser importance. He will be more than compensated by the quality of the information contained, which deals with those aspects of hepatic disease that are much more apt to concern him, including the description of the principles of treatment, both medical and surgical, by experts in the field. Furthermore, he will frequently find it unnecessary to consult other books, particularly on points dealing with basic concepts.

To various contributors the editor expresses his deep gratitude for their excellent and willing cooperation. He has considered it good fortune indeed to have been associated with them in this undertaking. He wishes to express his thanks to Cecil J. Waston, Arthur J. Patek, Jr., and to his colleague, Edward A. Gall, for their helpful suggestions.

Leon Schiff

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Preface

The editors trust that this 10th edition of *Diseases of the Liver* provides an accurate assessment of the many states of the art in hepatology. The platform on which hepatology is based has never been sturdier. The depth of understanding of many processes that take place in the liver and the multiple factors that affect them give us hope that ever more successful ways to diagnose and treat a variety of liver disorders is in the offing.

The clinical patterns and natural history of many liver diseases are now quite well described. The complex interactions that affect the course the individual patient with a liver disorder might follow are being assessed and to an ever-increasing extent understood. The 10th edition is introduced by a presentation of an approach to the evaluation of the patient with known or suspected liver disease utilizing history, physical examination, and widely available biochemical, serologic, and imaging studies. The 10th edition ends with a series of chapters on the present status of liver replacement. In between are remarkable chronicles of progress and the posing of questions yet to be answered. The foundations of hepatology based on clinical chemistry, imaging, and the power of properly interpreted liver biopsies are well described.

Considerable attention is paid to the hepatitis viruses and the consequences of infections with each. For hepatitis C, diagnostic and assessment methods are well established. The remarkable emergence of the story of hepatitis C is well told and embroidered in several chapters. The importance of hepatitis C genotype and the level of HCV RNA on outcome and likelihood of treatment success is emphasized. The ability to treat chronic hepatitis C has improved greatly during the last decade and new antiviral and immunomodulatory approaches are likely to emerge in the near future. What is missing for hepatitis C is an effective vaccine. The complex interactions between hepatitis C and other viruses have been recognized and the apparent synergistic effect of hepatic injury from hepatitis C and alcohol on fibrosis is an area receiving great attention.

Furthermore, interest in hepatitis B has never been higher. For hepatitis B, there are effective vaccines that have proved to be life

changing in areas of the world where programs that ensure availability and utilization of vaccines have been successful. However, there are millions of patients already infected with hepatitis B and many are destined to succumb to the complications of cirrhosis or hepatocellular carcinoma unless further progress in therapeutic development occurs.

The importation of ideas from basic science and many disciplines and clinical lessons learned from other specialties are apparent throughout the book. One area of interest clearly influences another. The role of viruses and the response to the presence of the viruses in the development of hepatocellular carcinoma is a fascinating field. There has been a veritable explosion of the number of cases of hepatocellular carcinoma, especially those related to hepatitis C over the last few years.

The ability of an individual to favorably respond to an environmental insult (be it a virus or a chemical) is attracting considerable interest. In drug-induced liver disease, early (innate) responses may well determine whether a patient has a transient perturbation in the liver or develops a devastating illness.

Then there is the focus on the many roles and consequences of fat in the liver. Once considered benign, fat deposition in the liver is now viewed as a potential cause of cirrhosis and even a precursor of hepatocellular carcinoma. The stories of the interactions of fat and fibrosis have overtones of genetic influences, as well as associations with other disorders grouped as the metabolic syndrome, which are clearly of growing importance.

The ever-increasing importance of liver transplantation is recognized in this edition, with a separate section devoted to topics of importance to the clinician caring for patients before and after liver transplantation. We have fashioned a book within a book regarding liver transplantation and trust this section will provide a comprehensive resource without the necessity to refer to more specialized references sources.

There is much more to be discovered and assimilated about the liver in the near future. Ways to modulate gene expression, understand (and even regulate) the many roles of nuclear receptors, the interactions of cytokines and hepatic growth factors, and the roles of metals and minerals— all these and many more areas are discussed in the 10th edition.

The editors and the authors are pleased to present this 10th edition. Differences are being made that will be life changing for patients with liver diseases. These are the best of times for those interested in the liver.

Eugene R. Schiff

Michael F. Sorrell

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Chapter 1

History Taking and Physical Examination for the Patient with Liver Disease

Norton J. Greenberger

Key Concepts

- In the care of patients with jaundice, a careful history, physical examination, and review of standard laboratory tests should allow a physician to make an accurate diagnosis in 85% of the cases.
- The triad of findings of splenomegaly, ascites, and an increased number of venous collateral vessels on the anterior abdominal wall indicates a diagnosis of portal hypertension.
- The presence of two physical findings (ascites and evidence of portal-systemic encephalopathy [asterixis]) and two laboratory findings (hypoalbuminemia [<2.8 g/dL] and a prolonged prothrombin time [>3 seconds]) indicates a diagnosis of cirrhosis of the liver.
- Three physical findings (parotid enlargement, gynecomastia, and Dupuytren's contracture) indicate that a patient is almost certainly consuming excessive amounts of alcohol.
- In adult patients with a new onset of jaundice, eight disorders account for 98% of the ultimately established diagnoses. They include viral hepatitis, alcohol-induced liver disease, chronic hepatitis (all causes), drug-induced liver disease, gallstones and their complications, carcinoma of the pancreas, primary biliary cirrhosis, and primary sclerosing cholangitis. By the time patients with metastatic liver disease have jaundice, the diagnosis should be obvious because the liver has been extensively replaced by tumor.

Jaundice is a common presentation among patients with liver and biliary

tract disease. The terms *jaundice* and *icterus* are used to designate skin and eyes appearing yellow resulting from the retention and deposition of biliary pigments (biliary monoglucuronides and diglucuronides). Although bilirubin stains all tissue, jaundice is most evident in the sclerae, face, and trunk. Jaundice is most commonly caused by parenchymal liver diseases such as viral hepatitis or cirrhosis, obstruction of the extrahepatic biliary tree as in choledocholithiasis and carcinoma of the pancreas, and less commonly, disorders associated with brisk hemolysis such as sickle cell anemia. The late Franz Ingelfinger stated in 1958 that the cause of jaundice can be identified in approximately 85% of patients after a careful study of the history and the performance of a physical examination and review of standard laboratory data. The same applies today. Table 1.1 lists the general and specific questions to ask in relation to the specific causes of liver and biliary tract disease.

History Taking for the Patient with Jaundice or Abnormal Results of Liver Tests

Anorexia is a cardinal symptom of viral hepatitis and of neoplasms involving the liver, colon, biliary tree, or pancreas. Weight loss of more than 10 pounds

(4.5 kg) should always raise the question of a neoplastic disorder.

Table 1.1. Specific Questions to Ask Patients with Jaundice or Liver Disease

RELATED TO VIRAL HEPATITIS

- Blood transfusions (especially if before 1990)
- Intravenous drug use
- Sexual practices
  - Anal-receptive intercourse
  - Sex with a prostitute
  - History of sexually transmitted disease
  - Multiple sexual partners (>5/y)
  - Intercourse with individuals with hepatitis B or C
- Contact with individuals with jaundice
- Changes in taste and smell
- Needlestick exposure
- Work in renal dialysis units
- Surgeons in trauma units or operating rooms exposed to users of intravenous drugs
- Shared razors or toothbrushes
- Body piercing (ears, nose)

Tattoos
Intranasal cocaine use

SPECIAL RISK FACTORS FOR HEPATITIS A

Travel to endemic areas
Ingestion of raw shellfish (harvested from contaminated waters)
Exposure to patients in places where clusters of hepatitis may occur (e.g., institutions, restaurants, preschool nurseries)

MEDICATION RELATED

Review all prescription medications
Ask specifically about all over-the-counter drugs
Ask specifically about vitamins (especially vitamin A)
Ask specifically about any foods, herbal preparations, home remedies purchased in a health food store

ALCOHOL USE

Obtain detailed *quantitative* history of both recent and previous alcohol use from the patient *and* family members
Question whether patient has experienced withdrawal symptoms, driving-under-the-influence convictions
CAGE (cut down, annoyed, guilty, eye opener) criteria (see text)
Check for evidence of alcohol-associated illnesses (pancreatitis, peripheral neuropathy)

MISCELLANEOUS QUESTIONS

Pruritus (suggests cholestasis either intrahepatic or extrahepatic)
Evolution of jaundice (dark urine, light stools)
Recent changes in menstrual cycle (amenorrhea suggests chronic liver disease, often cirrhosis)
History of anemia, sickle cell disease, known hemoglobinopathy, artificial heart valves
Symptoms suggestive of biliary colic, chronic cholecystitis
Family history of liver or gallbladder disease
History of inflammatory bowel disease (should raise the question of primary sclerosing cholangitis and receipt, if any, of blood transfusions)
Occupational history and, specifically, exposure to hepatotoxins

Chills and fever along with headache and myalgia should raise the question of viral hepatitis, especially type A. Chills and fever along with right upper quadrant abdominal pain suggest a diagnosis of biliary tract disease, especially choledocholithiasis and ascending cholangitis.

Arthritis can be the harbinger of viral hepatitis, autoimmune chronic

hepatitis, inflammatory bowel disease with underlying liver disease, primary sclerosing cholangitis, or granulomatous disorders such as sarcoidosis.

Fleeting skin lesions are often present in patients with viral hepatitis B. Excoriations, indicating pruritus, should raise the question of either intrahepatic or extrahepatic cholestasis, particularly primary biliary cirrhosis or primary sclerosing cholangitis. With regard to abdominal pain, the standard questions to ask concern the location, character, radiation, factors precipitating or relieving pain, and whether there are other systemic symptoms that accompany the pain. Patients should be asked to compare current abdominal discomfort with other causes of abdominal pain that they have experienced in the past (e.g., gastroesophageal reflux symptoms, non–ulcer-type dyspepsia).

Questions to be asked in relation to viral hepatitis include specific questions about blood transfusions, especially whether they were received before 1990.

The date is important because before that time no serologic tests were available for the detection of infection with hepatitis C virus. Intravenous drug use is currently the most common cause of hepatitis C. It is important to ask specifically about sexual practices, especially high-risk sexual behavior. In this regard, anal-receptive intercourse is known to be a significant risk factor for hepatitis B. Sexual practices associated with an increased risk of hepatitis C include a history of sexual relations with a prostitute, history of a sexually transmitted disease, and multiple sexual partners per year. In addition, intercourse with patients known to be positive for hepatitis B and C (e.g., spouses) is known to be a risk factor for contracting these forms of hepatitis. Contact with jaundiced individuals may be a risk factor for hepatitis A and B. Changes in taste and smell occur fairly frequently in patients with hepatitis, especially viral hepatitis A. They contribute, in a large part, to the anorexia experienced by such patients. This is in part due to a decreased sense of smell (hyposmia), perception of unpleasant smells from foods that are not ordinarily perceived as unpleasant (dysosmia), a decreased sense of taste (hypogeusia), and perception of unpleasant taste (dysgeusia). Hypogeusia is often reflected by the fact that patients may spontaneously state that they have lost their taste for cigarettes.

Health care professionals are at risk of contracting hepatitis C. This can occur through needlestick exposure, by working in renal dialysis units, and in trauma units, emergency departments, or operating rooms through surgical procedures on patients harboring the hepatitis C virus in whom that diagnosis is not immediately apparent. All users of intravenous drugs should be suspected of harboring the hepatitis C virus. Special risk factors for hepatitis C include tattoos, body piercing (e.g., of the ears and nose), history of snorting cocaine, and use of

shared razors or toothbrushes.

Special risk factors for hepatitis A include travel to endemic areas such as Mexico and Latin America and the African subcontinent, ingestion of raw shellfish that may have been harvested from contaminated waters, and exposure to patients in places where clusters of hepatitis may occur. The latter has been well documented in mental institutions, restaurants, preschool nurseries, and close living quarters. Hepatitis A can be transmitted parenterally because there is brief viremia.

Medication use, including all prescription drugs and all over-the-counter drugs, should be carefully reviewed. The constellation of clinical features that include fever, arthritis or arthralgia, rash, and eosinophilia in a patient with jaundice or abnormal results of liver tests should always raise the question of medication-induced liver disease. This can be recalled by the mnemonic FARE, which stands for fever, arthritis, rash, and eosinophilia. The patient should be asked specifically about intake of vitamins, especially vitamin A, and about any foods, herbal preparations, or home remedies purchased in health food stores. Several herbal preparations have been found to be hepatotoxic.

Detailed quantitative information should be obtained from both the patient and family members about recent and previous alcohol use. For reference purposes, 1 ounce (30 mL) of bourbon whiskey contains 10 to 11 g of alcohol, as does one 12-ounce (360 mL) container of beer or 4 ounces (120 mL) of red table wine. Each one of these can be considered as 1 unit. Ingestion of more than 3 units/day everyday or more than 21 units/week is excessive, especially for women. The threshold for alcohol-induced hepatic injury appears to be 30 g/day for women and 60 g/day for men if ingested over 5 to 10 years. These numbers may have to be modified if additional risk factors for liver disease are present (e.g., hepatitis C). One also needs to determine whether the patient has experienced withdrawal symptoms. The CAGE criteria are reliable indicators of excessive alcohol use. The CAGE criteria relate to the following four questions:

1. Has the patient tried to *c*ut back on alcohol use?
2. Does the patient become *a*ngry when asked about his or her alcohol intake?
3. Does the patient feel *g*uilty about his or her alcohol use?
4. Does the patient need an *e*ye opener in the morning?

In this regard, many patients with chronic alcoholism experience morning nausea and dry heaves. The examiner should check for evidence of alcohol-associated illnesses (e.g., pancreatitis and

peripheral neuropathy).

A history of pruritus suggests cholestasis, either intrahepatic or extrahepatic. Table 1.2 shows the differential diagnosis. The patient should be specifically asked about the evolution of jaundice (i.e., the onset of dark urine and light stools), which may provide clues to the duration of illness. Recent changes in the menstrual cycle, particularly amenorrhea, if present, suggests chronic liver disease and often cirrhosis. A history of anemia, sickle cell disease, and hemoglobinopathy should also be ascertained for African-American patients. Right upper quadrant abdominal pain should prompt detailed questions about whether such pain is consistent with biliary colic or chronic cholecystitis. A history of inflammatory bowel disease should raise the question of primary sclerosing cholangitis or, if the patient has received blood transfusions in the past, hepatitis C. An occupational history should be obtained and questions about specific exposure to a known or suspected hepatotoxin should be asked of industrial workers with jaundice or liver disease.

Table 1.2. Differential Diagnosis of Jaundice

MOST COMMON CAUSES
- Viral hepatitis
- Alcoholic liver disease
- Cholecystitis, choledocholithiasis
- Carcinoma of the pancreas

COMMON CAUSES
- Drug- or toxin-induced liver disease
- Chronic hepatitis
- Sickle cell anemia
- Sepsis
- Postoperative state
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

LESS COMMON CAUSES
- Hodgkin's disease, non-Hodgkin's lymphoma
- Total parenteral nutrition
- Gilbert's syndrome (unconjugated hyperbilirubinemia rarely exceeds 3.0 mg/dL and detectable jaundice is infrequent)
- Metastatic liver disease (jaundice does not develop until >85%–90% of the liver is replaced by tumor)

CAUSES AND PRESUMED SITES OF INTRAHEPATIC CHOLESTASIS
- Liver cell (hepatocellular)
  - Viral hepatitis

- Alcoholic liver disease
- Chronic active liver disease
- α_1-Antitrypsin deficiency

Hepatocanalicular
- Drugs (androgens, phenothiazines)
- Sepsis
- Postoperative state
- Total parenteral nutrition
- Hodgkin's and non-Hodgkin's lymphoma
- Amyloidosis
- Sickle cell anemia
- Toxic shock syndrome

Ductular
- Sarcoidosis
- Primary biliary cirrhosis

Bile ducts
- Intrahepatic biliary atresia
- Caroli's disease
- Cholangiocarcinoma
- Primary sclerosing cholangitis

Recurrent cholestasis
- Benign recurrent intrahepatic cholestasis
- Recurrent jaundice of pregnancy
- Dubin-Johnson syndrome

Physical Examination of the Patient with Jaundice or Abnormal Results of Liver Tests

The key elements in the physical examination of a patient with jaundice or abnormal results of liver tests are summarized in Table 1.3. Several important clues are evident on general inspection of the patient. It should be determined whether scleral icterus is present, and this should be done in natural daylight. Scleral icterus can usually be detected if the serum bilirubin level is elevated to values greater than 3.0 mg/dL. The presence of pallor suggests anemia. Wasting suggests advanced chronic liver disease or a neoplastic disorder. Needle tracks or evidence of skin popping suggest intravenous drug abuse. The presence of skin excoriation confirms that the patient has been experiencing pruritus, which can be particularly severe among patients with primary biliary cirrhosis and primary sclerosing cholangitis. The one area where such patients cannot scratch is the interscapular area, and this is usually free of evidence of excoriation. The presence of ecchymosis or petechiae raises the question of clotting problems, especially

thrombocytopenia.

Muscle tenderness and weakness are not uncommon among patients with chronic alcoholism and alcoholic myopathy. These findings are often overlooked. When associated with severe acute pancreatitis, discoloration of the abdomen is termed the *Grey Turner's sign.* This finding implies increased likelihood of death. Other less common causes of ecchymosis include rhabdomyolysis, muscle infarction, mesentery thrombosis, strangulated bowel with extensive intestinal infarction, and massive intraperitoneal bleeding.

Table 1.3. Physical Examination of the Patient with Jaundice

GENERAL INSPECTION
- Scleral icterus
- Pallor
- Wasting
- Needle tracks
- Evidence of skin excoriations
- Ecchymosis or petechiae
- Muscle tenderness and weakness
- Lymphadenopathy
- Evidence of pneumonia
- Evidence of congestive heart failure

PERIPHERAL STIGMATA OF LIVER DISEASE
- Spider angiomata
- Palmar erythema
- Gynecomastia[a]
- Dupuytren's contracture[a]
- Parotid enlargement[a]
- Testicular atrophy
- Paucity of axillary and pubic hair
- Eye signs mimicking hyperthyroidism

ABDOMINAL EXAMINATION
- Hepatomegaly
- Splenomegaly
- Ascites
- Prominent abdominal collateral veins
- Bruits and rubs
- Abdominal masses
- Palpable gallbladder

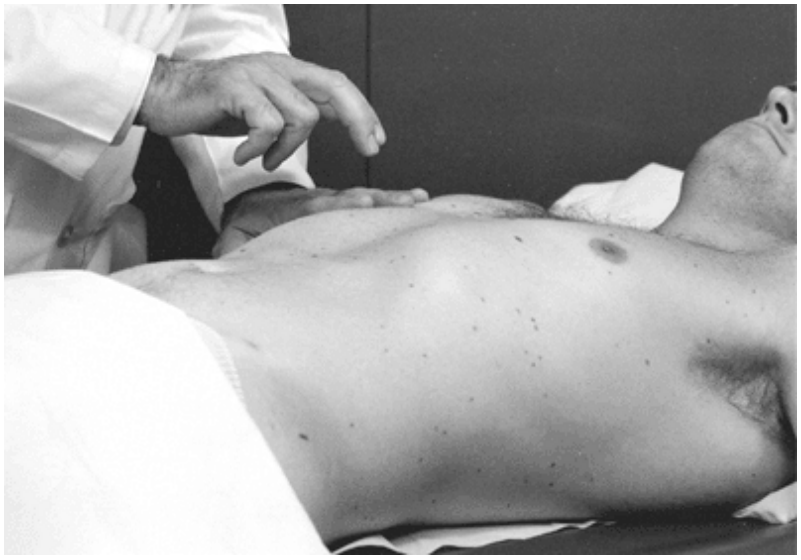
SIGNS OF "DECOMPENSATED" HEPATOCELLULAR DISEASE
- Jaundice
- Ascites

Oliguric hepatic failure
Hepatic encephalopathy
Fetor hepaticus
Asterixis
Behavioral alterations (confusion, disorientation, failure to complete simple mental tasks)

[a]This triad suggests chronic alcoholism.

The presence of lymphadenopathy, if generalized, suggests a lymphoproliferative disorder such as Hodgkin's disease or non-Hodgkin's lymphoma. Supraclavicular lymphadenopathy should raise the question of underlying malignant disease of the stomach or bronchopulmonary tract. Patients with pneumonia have jaundice in approximately 5% of cases; this is more likely to be the case among patients with pneumococcal pneumonia. Accordingly, a careful examination of the lungs is in order in the evaluation of patients with jaundice. Patients with congestive heart failure quite frequently have chronic passive congestion of the liver, which can result not only in jaundice but also in signs of portal-systemic encephalopathy.

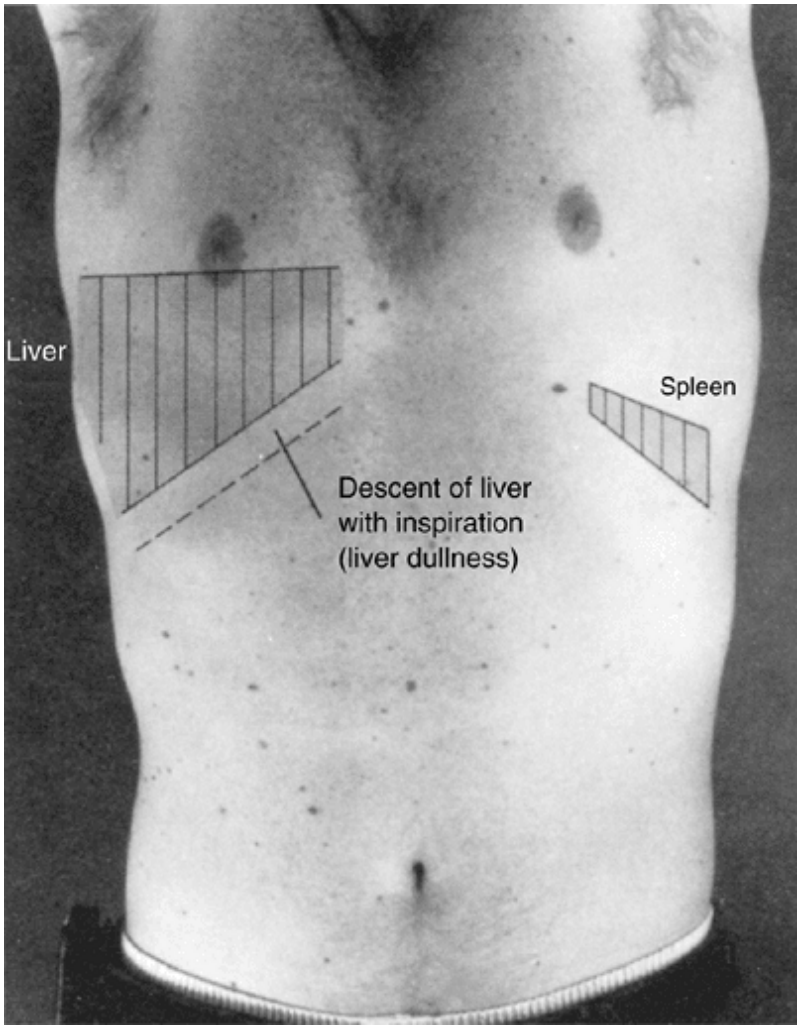
There are several peripheral stigmata of chronic parenchymal liver disease. Spider angiomata are usually found in the distribution of the superior vena cava and most commonly on the upper anterior chest, neck, face, and upper thorax. The presence of more than a dozen spider angiomata should raise the question of portal hypertension. The triad of gynecomastia, Dupuytren's contracture, and parotid enlargement should always raise the question of chronic alcoholism. Paucity of axillary and pubic hair and eye signs mimicking those of hyperthyroidism are often found among patients with advanced liver disease. Testicular atrophy, defined by a testicular diameter of less than 3 cm, is also common.



▪ **Figure 1.1** Determination of liver size by means of percussion over the lower right anterior chest and the right upper quadrant of the abdomen.

The abdominal examination is important in determining the liver size as well as the presence of an enlarged spleen. Percussion of the abdomen is important for several reasons. First, the size of solid organs such as the liver and spleen can be evaluated with percussion. One can often determine whether an increased amount of intraperitoneal fluid (ascites) is present. The upper and lower borders of liver dullness can be assessed by means of percussion along the right midclavicular line from the midchest to the midabdomen (Fig. 1.1). Liver size can be further assessed by having the patient inspire and observing the descent of the liver (Fig. 1.2). The lower border of liver dullness alerts the examiner to the site where the liver edge should be palpable. The liver span as judged by liver dullness measures 10 to 12 cm in men and 8 to 11 cm in women. A sudden decrease in liver dullness can occur in several conditions, such as viral hepatitis with the development of submassive or massive liver cell necrosis, localized dilatation of the transverse colon (as in toxic megacolon), fulminant colitis, and ileus associated with peritonitis or a perforated viscus (e.g., duodenal ulcer or diverticulitis). The spleen is normally

not palpable. Percussion over the spleen reveals an area of dullness extending from the 10th rib in the posterior midaxillary aspect to the anterior aspect of the chest (Figs. 1.2 and 1.3). When the patient inspires, the area of splenic dullness moves inferiorly and to the right.



▪ **Figure 1.2** Photograph of abdomen and chest depicts the location of the liver and the spleen as outlined by means of percussion. The liver descends 1 to 3 cm with inspiration, which is reflected by a change in liver dullness.

The detection of splenic dullness is important for the following three reasons:

1. It may indicate splenic enlargement before the spleen can be palpated.
2. It alerts the examiner to the site where the spleen may be palpated.
3. Increasing dullness of the left flank may be a valuable clue to the diagnosis of traumatic rupture of the spleen or subcapsular hematoma.

All quadrants of the abdomen must be palpated in an orderly manner. When palpating the abdomen, the hand should be warm and the palm and extended fingers of the right hand placed flat in a plane parallel to the surface of the abdomen (Fig. 1.4). The pads of the fingers are used together to perform light general palpation. Light palpation is used on the abdomen first, and as tense muscles relax, deeper palpation should be tried. Quick jabbing movements should be avoided. Any area of tenderness or any increased muscular resistance should be recognized and examined in detail.

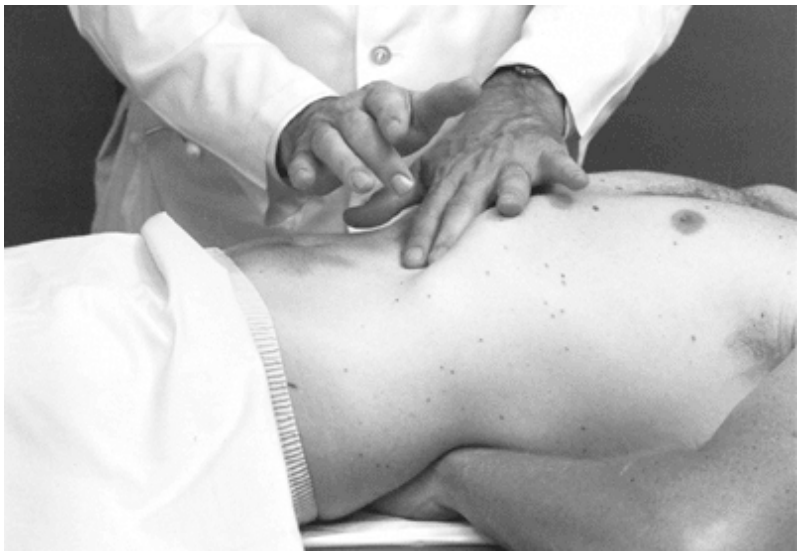
Percussion should alert the examiner to the approximate size and lower edge of the liver. Beginning at the right iliac fossa, the right hand is moved gradually upward until the edge of the liver is appreciated (Fig. 1.4). The patient can be asked to take a deep breath slowly. The descent of the diaphragm carries the liver down, which facilitates palpation of the liver edge. The edge of the liver can be felt in most healthy individuals if the patient's abdominal wall muscles are relaxed and the patient takes a slow, deep breath. In some healthy individuals, a very low lying thin segment of the liver can be palpated in the right lower quadrant. This is termed the *Riedel lobe of the liver.* An alternative approach to feeling the edge is to gently curl the fingers of the right hand below the costal margin and ask the patient to inspire slowly (Fig. 1.5). This is termed the *Middleton method.* In this manner, the liver descent is appreciated by the fingertips. This method is important in determining minimal enlargement of the liver or a liver palpable only in the epigastrium, as can occur in advanced cirrhosis.

The examiner should determine whether the liver is soft, firm, hard, or irregular; whether the edge is rounded or sharp; whether discrete masses are present; and whether the left lobe is palpable across the midline. The presence of a palpable left lobe always denotes an abnormality, usually chronic liver disease. The size of the liver should be assessed as judged by the location of the edge below the right midclavicular line and the xiphoid. A normal liver edge is sharp, smooth, and not hard, and the left lobe is not palpable. A rounded edge suggests liver disease; a palpable left lobe suggests either chronic infiltrative or neoplastic liver disease. Modest enlargement of the liver occurs in several disorders, most notably viral hepatitis, chronic liver disease (all causes), chronic hepatitis, cirrhosis, choledocholithiasis, and extrahepatic biliary tract obstruction. Marked enlargement of the liver (edge >10 cm below the costal margin) occurs in relatively few disorders, which include (a) primary and metastatic tumors of the liver, including lymphoma, (b) alcoholic liver disease (fatty liver, alcoholic hepatitis, cirrhosis), (c) severe congestive heart failure, (d) infiltrative diseases of the liver, such as amyloidosis and myelofibrosis, and (e) chronic myelogenous leukemia. Finally, a pulsatile liver should raise the question of tricuspid regurgitation, which can occur with advanced mitral stenosis, endocarditis of the tricuspid valve, and severe

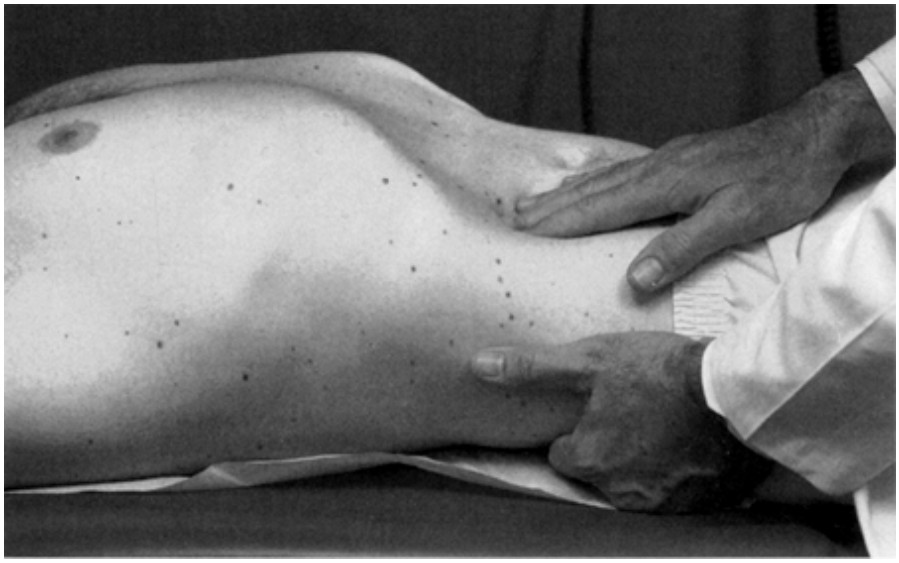
pulmonary hypertension.

Percussion of the left upper quadrant may have alerted the examiner to the presence of an enlarged spleen. Palpation of the spleen should begin in the left iliac fossa and move up to the left costal margin (Fig. 1.6). If the spleen is not felt while the patient is supine, the patient should roll onto his or her right side so the examiner can again examine the left upper quadrant (Fig. 1.7).

This method takes advantage of the fact that when the spleen enlarges, it becomes more easily palpable inferiorly and medially. This enlargement is better appreciated when the patient is in the right lateral decubitus position. An alternative is for the examiner to stand at the patient's right side with the patient's left hand placed under the 11th rib to elevate the thorax. The examiner then curls the fingers of either one or both hands below the costal margin and asks the patient to inspire. The splenic margin may then be felt by the fingertips.



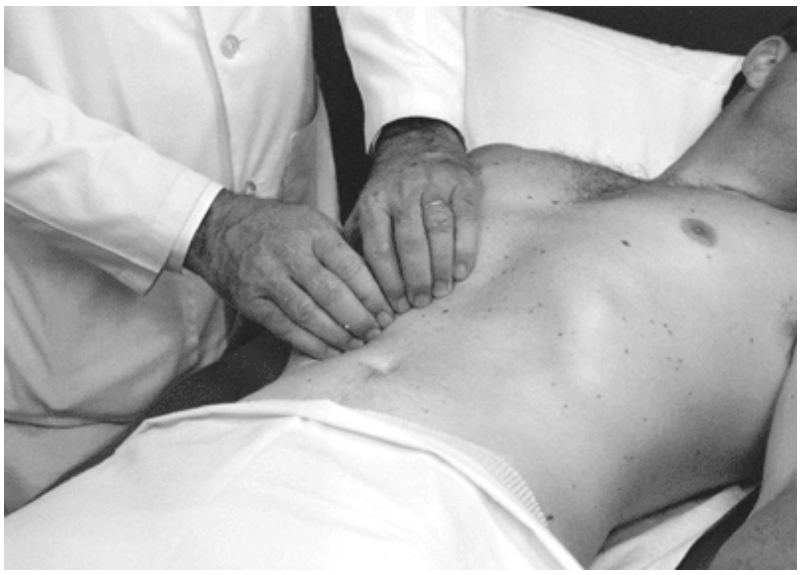
▪ **Figure 1.3** Technique for percussion of the spleen. If splenomegaly is present, the percussion note is dull, and with inspiration, the spleen moves downward and medially and the percussion note changes accordingly.



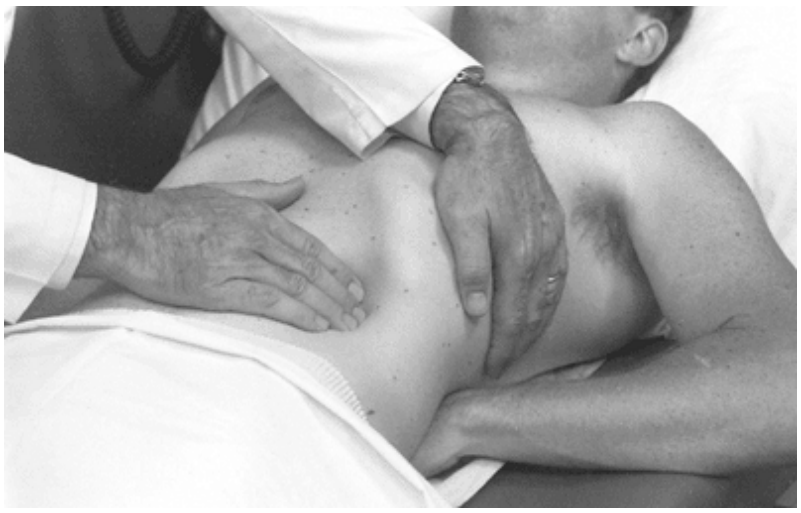
▪ **Figure 1.4** Technique for palpation of the liver.

A common problem in evaluating left upper quadrant masses is differentiating the spleen and the left kidney. Palpation of a notch on the medial surface

suggests that the organ being palpated is the spleen. Differentiation of the left lobe of the liver from the spleen can be difficult if massive hepatomegaly is present. One can usually discern a space or open area between the two organs. Common causes of splenomegaly include the following:

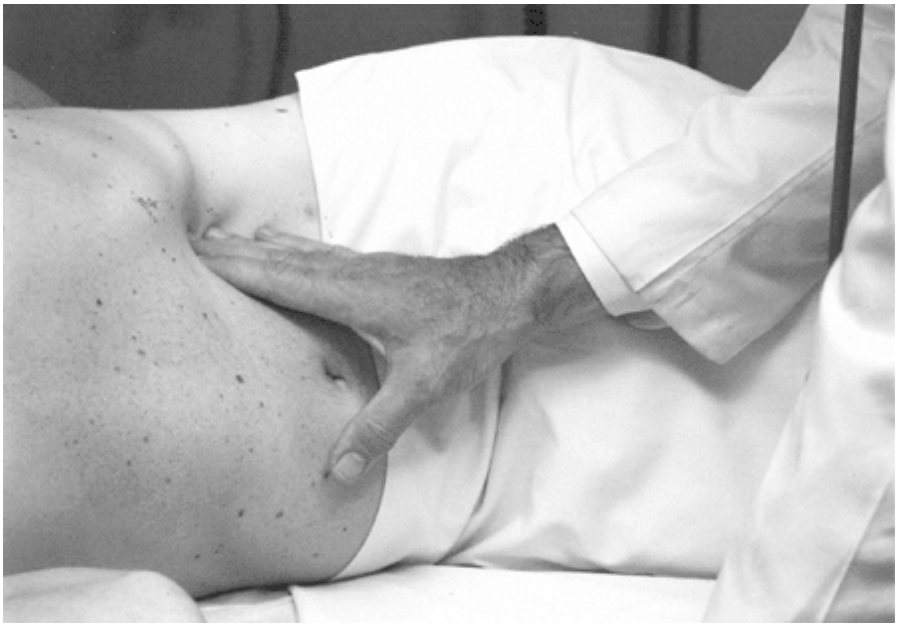


▪ **Figure 1.5** Alternative technique for palpation of the liver. The best results are obtained with gentle pressure of the curled fingers on the anterior abdominal wall.



▪ **Figure 1.6** Technique for palpation of the spleen.

1. Portal hypertension caused by cirrhosis of the liver
2. Infections (viral, bacterial, fungal)
3. Leukemia, lymphoma, and Hodgkin's disease
4. Connective tissue diseases (systemic lupus erythematosus and rheumatoid arthritis)
5. Infiltrative disorders (amyloidosis and sarcoidosis)



▪ **Figure 1.7** Palpation with the patient in the right lateral decubitus position should be performed on all patients with suspected splenomegaly if the spleen is not felt with the patient in the supine position.

6. Hemolytic disorders
7. Myelofibrosis

Gallbladder

The gallbladder, when enlarged, can often be palpated in the right upper quadrant at the angle formed by the lateral border of the rectus abdominis muscle and the right costal margin. The gallbladder is palpable in approximately 25% of cases of carcinoma of the head of the pancreas (Courvoisier's law) because of painless distention of the gallbladder. The gallbladder is also palpable in approximately 30% of patients with acute cholecystitis, often because stones are impacted in the neck of the gallbladder. Often in acute cholecystitis, rather marked right upper quadrant tenderness is present, and palpation can be difficult because of intense involuntary spasms of the abdominal muscles. Percussion over the right lower anterior chest and right upper quadrant often elicits pain.

Another sign pointing to acute cholecystitis is right upper quadrant abdominal pain aggravated by inspiration (Murphy's sign). The patient is asked to inspire after the examining fingers are placed high in the

right upper quadrant; inspiration causes the gallbladder to descend and come in contact with the extended fingers, causing pain and inspiratory arrest.

Bruits

Bruits are systolic sounds usually produced by the turbulence of blood flowing through diseased or compressed blood vessels. The many causes of abdominal bruits are listed in Table 1.4. The most common causes of such bruits include calcification of the aorta, celiac axis compression, and alcoholic hepatitis. An epigastric bruit can be appreciated in 20% of healthy thin young adults, especially if auscultation is performed after a meal. Such bruits are usually caused by the compression of the celiac axis artery by muscle fibers of the crus of the diaphragm. Abdominal bruits are often important clues leading to the diagnosis of hepatocellular carcinoma, renal artery stenosis, fibromuscular hyperplasia of the renal arteries, intestinal angina, aortic aneurysm, and pancreatic cancer.

Peritoneal Friction Rub

A friction rub heard over the liver suggests the diagnosis of liver metastasis or primary hepatocellular carcinoma. Other causes of hepatic friction rub include infarction of the liver (as in sickle cell anemia and polyarteritis nodosa) and liver abscess. A transient friction rub caused by a hematoma around the puncture site is common after liver biopsy but is usually not audible 4 to 6 hours after biopsy.

Table 1.4. Causes of Abdominal Bruit

Location of bruit	Diagnostic considerations	Comment
Liver	Alcoholic hepatitis	Bruits may change day to day
	Hepatocellular carcinoma	Suspect hepatocellular carcinoma in decompensated cirrhosis with a disproportionately increased serum level of alkaline phosphatase
	Effect of surgery	

	Portosystemic shunt	
	Hepatic artery aneurysm	
	Hepatic arteriovenous fistula (trauma)	
Spleen	Splenic artery aneurysm	May see calcification in left upper quadrant on plain radiographs of the abdomen
	Splenorenal shunt	
	Splenic arteriovenous fistula	
	Calcified aorta	
Aorta	Aortic aneurysm	
	Celiac axis compression	Bruits common in thin individuals, especially after meals
	Celiac or superior mesenteric artery disease (atheroma, thrombi)	Intestinal angina characterized by the triad of (a) bruit, (b) weight loss, and (c) postprandial abdominal pain
Left upper quadrant	Pancreatic cancer (body or tail)	Bruit caused by encasement of splenic artery or vein by tumor;

		present in 25% of cases
Umbilicus	Cruveilhier-Baumgarten syndrome	Bruit caused by increased flow through umbilical veins secondary to portal hypertensions
	Renal artery stenosis (atheroma, emboli)	
	Renal artery fibromuscular hyperplasia	Bruit may be unilateral or bilateral
	Renal artery aneurysm	

Ascites

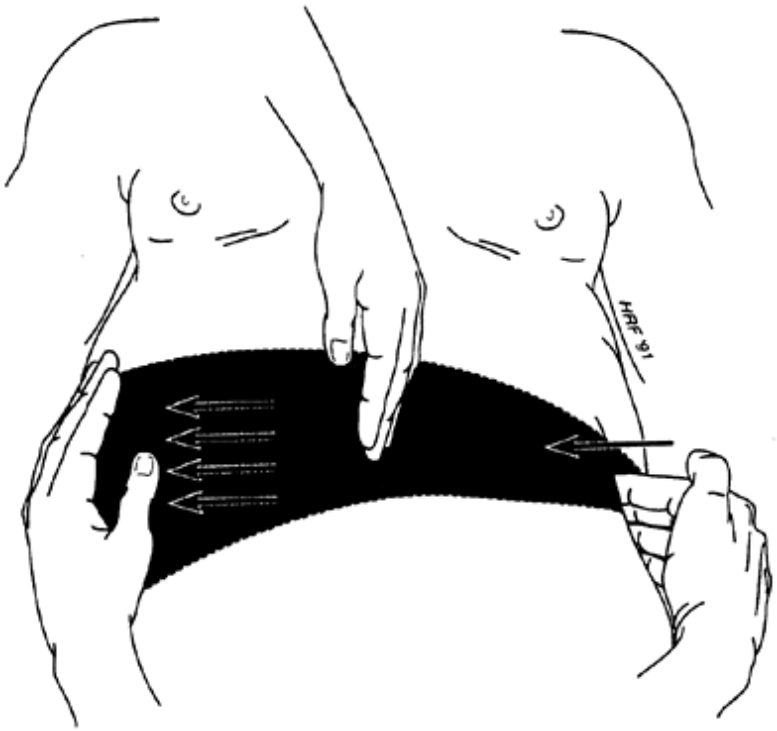
Assessment of shifting dullness is often used to determine whether ascites is present. When free fluid is present in the abdomen, such fluid gravitates to the flanks, and the intestines float upward when the patient lies on his or her back. Percussion with the patient in this position discloses tympany over the anterior abdomen and dullness over the flanks. If the patient is turned to one side, the dullness shifts, and the percussion on the side that is uppermost becomes tympanic, because that area becomes occupied by gas-filled intestine.

Another physical finding pointing to the diagnosis of ascites is the fluid wave. A fluid wave is demonstrated by tapping the left flank sharply with the right hand while the left hand is placed against the opposite flank (Fig. 1.8). Either the patient or a second examiner must place the ulnar surface of his or her hand along the midline of the abdomen. A positive test result is one in which an impulse on the opposite flank is percussed after the right flank is tapped. Neither the test for shifting dullness nor the test for a fluid wave uniformly reveals modest amounts of ascitic fluid (<1,000 mL). Indeed, both tests have a sensitivity of only approximately 60% compared with ultrasound examination. Furthermore, the test results can be spuriously positive in examinations

of obese patients.

A third sign, bulging flanks, although often present in ascites, is frequently present in obese patients as well. A fourth test of ascites is elicitation of the puddle sign. In this test, the patient lies prone for a few minutes and then moves to hands and knees. The diaphragm of the stethoscope is placed over the most dependent part of the abdomen, where puddling would be expected

to occur. The examiner then repeatedly flicks the near flank of the abdomen while moving the stethoscope toward the opposite flank. A positive test result consists of a definite change in the intensity and character of the percussion note as the stethoscope is moved.



▪ **Figure 1.8** Technique for eliciting a fluid wave. The examiner's left hand is placed on the right lateral abdomen, and the right hand taps the left flank or loin while a second examiner or the patient's hand compresses the abdomen in the midline.

The presence or absence of ascites can be confirmed most reliably with imaging procedures such as ultrasonography and computed tomography (CT). The urinary bladder is often percussed in the hypogastrium, especially if urinary retention is present.

Abdominal Masses

All nine areas of the abdomen should be palpated carefully for the presence of abdominal masses. In addition, the examiner should palpate the periumbilical area for the presence of lymph nodes. Such lymph nodes, termed Sister Mary Joseph's nodes, reflect intraperitoneal tumor and are the harbinger of peritoneal carcinomatosis.

Signs and Symptoms of Decompensated Liver Disease

Signs of decompensated liver disease in patients with cirrhosis include jaundice, ascites, portal hypertension with bleeding esophageal or gastric varices, oliguric hepatic failure, and hepatic encephalopathy. These are discussed in detail in Chapters 11, 13, and 18. A bedside diagnosis of cirrhosis of the liver can be made on the basis of two physical findings and two laboratory findings. The physical findings are ascites and asterixis, and the laboratory findings are a serum albumin level of less than 2.8 g/dL and a prolongation of the prothrombin time of more than 16 seconds.

The topic of hepatic encephalopathy is discussed in detail in Chapter 18. However, this diagnosis can be made readily at physical examination when the following findings are present:

1. Hypothermia with a temperature less than 36°C
2. Fetor hepaticus, which is a pungent odor in the breath caused by the excretion of sulfur-containing amino acid by-products such as dimethyl sulfide, methanethiol, and ethanethiol
3. Asterixis, which can be elicited by two techniques (Fig. 1.9), and abnormal cognitive functioning as evidenced by an abnormal result of a Reitan trial test or A-deletion test. The latter is performed by asking the patient to delete all the A's in one or two paragraphs of newsprint; the examiner tabulates how many A's were not deleted.

Asterixis, although characteristic of hepatic encephalopathy, is not pathognomonic of this disorder. Asterixis is also found in patients with renal failure, pulmonary insufficiency, and congestive heart failure. Patients with hepatic encephalopathy may have difficulty with seemingly simple tasks such as drawing a square, spiral, or five-cornered star or signing their names.

The diagnosis of chronic portal-systemic encephalopathy is usually established on the basis of the following five criteria:

1. Documented chronic parenchymal liver disease
2. Evidence of portasystemic shunting, occurring either naturally (varices) or following the insertion of a portacaval shunt or

transjugular intrahepatic portasystemic shunt

3. Behavioral alterations that can range from subtle abnormalities in cognitive functioning to frank unresponsive coma
4. An abnormal result of an electroencephalogram showing a decrease in mean cycle frequency
5. Improvement in mental status after measures directed at altering gastrointestinal ammonia metabolism, such as with dietary protein restriction or lactulose therapy.

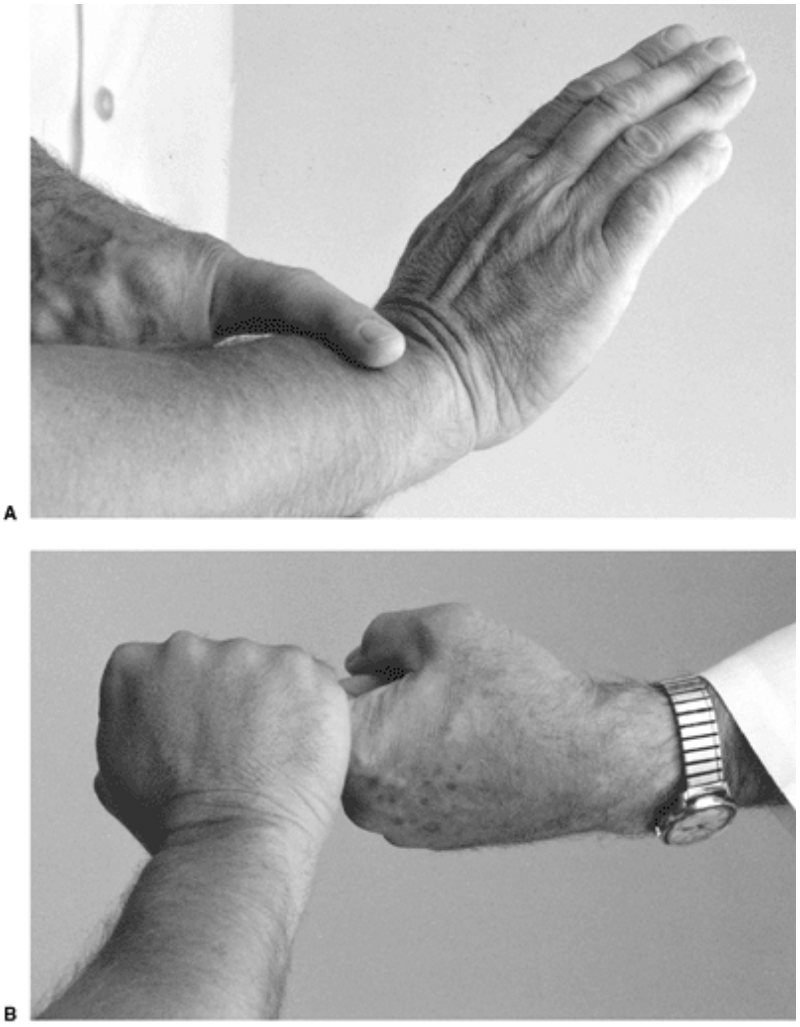
Role of Noninvasive Imaging

Role of Noninvasive Imaging of the Liver and Biliary Tree in the Evaluation of the Patient with Jaundice and/or Liver Disease

As noted earlier, in patients with jaundice, a careful history, physical examination, and review of standard laboratory tests should allow a physician to make an accurate clinical diagnosis in 85% of cases. All too often, however, an imaging procedure is done before these basic steps have been completed and such omissions may actually delay establishing the correct diagnosis. The following are offered as guidelines to be considered for ordering imaging procedures:

1. Liver imaging procedures are not indicated in a patient with acute viral hepatitis.
2. Liver imaging procedures are not necessary in a patient with a firm clinical diagnosis of nonalcoholic fatty liver disease.
3. In a patient with jaundice and obstructive-type liver test abnormalities, that is, a disproportionately elevated serum alkaline phosphatase level and serum aminotransferases less than 300 units, ultrasonography is the usual initial imaging procedure. Ultrasonography is reliable for differentiating medical, that is, intrahepatic cholestasis from extrahepatic obstructive jaundice. In a patient with a serum bilirubin greater than 10 mg/dL that has been present for more than 2 weeks, ultrasound has a

sensitivity of 95% and a specificity of 95% in differentiating intrahepatic versus extrahepatic causes of jaundice. Table 1.2 lists several causes of intrahepatic cholestasis. In some cases, the level and cause of bile duct obstruction can be identified.



▪ **Figure 1.9** Techniques for eliciting asterixis. **A:** The examiner applies his index finger over the dorsum of the patient's wrist while asking the patient to dorsiflex the wrist. Asterixis is the downward drift and abnormal recovery motion of the hand with the fingers either together or outstretched. **B:** Alternative method for eliciting asterixis in patients who may not have the requisite extensor tonus. The examiner asks the patient to clench his fingers around the examiner's fingers. Asterixis is elicited with subtle movements of the examiner's wrist. In this manner, one can both feel and see the asterixis movement.

4. If an ultrasound examination in a patient with jaundice reveals evidence of dilated intrahepatic ducts, additional imaging procedures such as CT scanning, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), and endoscopic ultrasonography

(EUS) with biopsies are often necessary to establish the cause of obstruction. If no dilated ducts are found and the cause of jaundice is deemed to be "medical," a liver biopsy is frequently in order.

5. In patients with suspected cholelithiasis, ultrasonography is the primary diagnostic imaging procedure. In patients with suspected choledocholithiasis and especially if there is a question of ascending cholangitis, ERCP is the preferred diagnostic procedure. In addition, ERCP with papillotomy may be indicated.
6. In a patient with new onset of ascites, Doppler ultrasonography is indicated to determine the potency of the portal and hepatic venous systems.
7. In patients with obstructive jaundice due to suspected pancreatic carcinoma, EUS is superior to CT scanning with regard to detecting a pancreatic mass but comparable with regard to tumor staging and predicting either respectability or nonrespectability.
8. In patients with unexplained hepatomegaly, either ultrasound or CT scanning are appropriate initial imaging procedures.

Case Vignettes: Practical Considerations

The following are vignettes in which the salient features of a patient's illness alert the clinician to the most likely diagnosis.

1. A 35-year-old woman is referred with the diagnosis of acute viral hepatitis. Liver tests reveal a total serum bilirubin level of 3.0 mg/dL; serum aspartate aminotransferase (AST), 900 U/L; serum alanine aminotransferase (ALT), 880 U/L; serum alkaline phosphatase, 180 U/L; serum albumin, 3.6 g/dL; serum globulins, 5.8 g/dL; prothrombin time, 15 seconds (international normalized ratio [INR], 1.9).

   Key: The presence of marked hypergammaglobulinemia along with prolongation of prothrombin time suggests that this patient has *chronic* rather than acute hepatitis. The most likely diagnosis for this 35-year-old woman would be autoimmune chronic hepatitis.

2. A 30-year-old man is referred for evaluation of elevated serum aminotransaminase levels. The patient weighed 230 pounds (103.5 kg) as a high school football player, 250 pounds (112.5 kg) after graduating from college, and 300 pounds (135 kg) 5 years later. Results of all liver tests are normal except for a serum AST level of 65 U/L and a serum ALT level of 80 U/L.

   Key: If a patient has no symptoms except that the transaminase

values are elevated approximately twice the normal value, six considerations should come to mind. They include (a) excessive alcohol use, (b) obesity with body mass index (BMI) greater than 32, (c) hepatitis C, (d) excessive doses of acetaminophen (>4.0 g/day), and (e) use of statin-type cholesterol-lowering agents and (+) celiac sprue. In this patient, the most likely explanation is obesity, and he may well have nonalcoholic steatohepatitis.

3. A 30-year-old man has severe flu symptoms for 5 days. Cough, myalgia, headache, and anorexia have resulted in markedly diminished intake of food and liquid. He has been ingesting generic acetaminophen 4.0 g/day and acetaminophen plus diphenhydramine (Tylenol PM) to facilitate sleep. He was found to have a serum AST level of 5,000 U/L and an ALT level of 5,500 U/L.

   Key: Ingestion of seemingly therapeutic doses of acetaminophen by a patient who has in essence fasted for 4 or 5 days can result in drug-induced hepatotoxicity. Results of early studies indicated that concurrent ingestion of alcohol and acetaminophen was the most common cause of this presentation. However, the scenario in this case is the more common presentation now. In patients who come to medical attention with serum aminotransaminase values of approximately 5,000 U/L, the differential diagnosis is limited to viral hepatitis, effects of drugs and toxins, and ischemia with shock liver. In the United States, approximately one half of cases of fulminant hepatic failure are related to acetaminophen ingestion.

4. A 63-year-old woman with non–insulin-requiring diabetes mellitus and hypercholesterolemia has been found to have abnormal results of liver tests. Results of liver biopsy establish the diagnosis of cirrhosis, but there is no obvious cause, and the patient is considered to have cryptogenic cirrhosis.

   Key: A detailed history reveals that the patient was obese through much of her adult life, weighing in excess of 300 pounds (135 kg; BMI >32). Although she weighed only 200 pounds (90 kg) when she sought medical attention, many years of obesity coupled with diabetes and hypercholesterolemia most likely caused nonalcoholic steatohepatitis, which progressed to cirrhosis. The most common cause of cryptogenic cirrhosis is antecedent nonalcoholic steatohepatitis.

5. A 28-year-old man who has had ulcerative colitis for 10 years is found through routine screening to have a serum alkaline phosphatase level of 500 U/L. Results of all other liver tests are normal.

   Key: Persistent elevation of serum alkaline phosphatase level to

values greater than twice the normal value in patients with inflammatory bowel disease, especially those with ulcerative colitis, should raise the question of occult primary sclerosing cholangitis. This patient needs either MRCP or ERCP.

6. A 40-year-old woman is admitted to the hospital for therapy for alcohol withdrawal and impending delirium tremens. Laboratory studies reveal serum bilirubin, 2.0 mg/dL; AST, 225 U/L; serum ALT, 45 U/L; prothrombin time, 14 seconds (INR, 1.3); serum iron level 170 µg/dL; total iron-binding capacity, 200 µg/dL; iron saturation, 85%; and serum ferritin, 700 µg/dL.

   Key: The increased percentage of iron saturation and elevated serum ferritin level in this case are not indicative of a diagnosis of hemochromatosis. Serum iron levels and serum ferritin levels are frequently elevated in patients with acute liver disease and marked liver cell necrosis.

7. A 17-year-old boy is referred for evaluation of hepatosplenomegaly and thrombocytopenia. On examination, he is found to have a peculiar "wing-beating" tremor. When the patient abducts his arms and flexes his forearms, a sustained tremor develops. The patient does not have Kayser-Fleischer rings. The serum ceruloplasmin level is 30 mg/dL.

   Key: The presence of a wing-beating tremor in a young man with apparent chronic liver disease is almost pathognomonic of Wilson disease. Examination of a 24-hour urine collection for copper revealed excretion of 1,100 µg of copper. Of the three standard screening tests for Wilson disease, that is, Kayser-Fleischer rings, low serum ceruloplasmin level, and increased urinary excretion of copper, results of all three are abnormal in only one third of patients. In the remaining two thirds of patients, only one test may have a positive result. The clue in this case was the unusual tremor, which is characteristic of Wilson disease.

8. A 25-year-old Guatemalan man who does not speak English is visiting relatives in the United States. After a large meal, he develops retching and hematemesis. Esophagogastroduodenoscopy reveals esophageal varices. The patient is admitted to the hospital and found to have hepatosplenomegaly, thrombocytopenia, a normal serum albumin level, and minimal hyperbilirubinemia (serum bilirubin, 2.0 mg/dL). Through interpreters, it is learned that the patient had had his first episode of hematemesis at 10 years of age. He had several subsequent episodes managed by means of banding of varices.

   Key: The onset of variceal bleeding at 10 years of age with stigmata of chronic liver disease but with well-preserved liver

function indicates that the most likely diagnosis is congenital hepatic fibrosis.

9. A 55-year-old man is referred for evaluation of asymptomatic hepatosplenomegaly. The patient's liver spans over 20 cm with a smooth, rounded edge felt 14 cm below the costal margin with a prominent left lobe. The spleen is palpable 4 cm below the left costal margin. Serum bilirubin, alkaline phosphatase, AST, and ALT levels are normal. The serum albumin level is 3.5 g/dL and serum globulin levels, 3.0 g/dL. Prothrombin time and partial thromboplastin time are normal. Results of a complete blood cell count and routine chemical analyses are normal. Urinalysis is unremarkable except for 2+ proteinuria. Blood glucose levels are normal, and the patient is not overweight.

   Key: The finding of marked hepatomegaly with normal results of liver tests in the evaluation of a patient without symptoms should raise the question of infiltrative liver disease, such as amyloidosis. In addition to amyloidosis, relatively few disorders give rise to marked hepatomegaly. These include primary and metastatic tumors of the liver, alcoholic liver disease, severe congestive heart failure, and advanced chronic myelogenous leukemia.

10. A 49-year-old man has documented end-stage chronic liver disease due to hepatitis C and alcohol ingestion. He has had previous hospital admissions for bleeding esophageal varices and ascites. Because of lack of compliance with a salt- and fluid-restricted diet, the patient has recurrent ascites despite treatment with diuretics. He is known to have diverticular disease, having had one previous episode of diverticulitis. He is admitted to the hospital with a 2-day history of severe abdominal pain, chills, fever, and anorexia. Diagnostic paracentesis reveals the following: White blood cell count, 10,000/μL with 90% polymorphonuclear leukocytes; ascitic fluid protein, 4.0 g/dL; ascitic fluid glucose, 35 mg/dL with a simultaneous serum glucose level of 120 mg/dL and peritoneal fluid of pH 7.1.

    Key: This patient does not have spontaneous bacterial peritonitis. Rather, he has *primary* bacterial peritonitis. The triad of findings of a white blood cell count of 10,000/μL with a left shift, increased serum protein level, and low glucose level points to primary bacterial contamination of the peritoneal cavity, rather than spontaneous bacterial peritonitis, most likely from a perforated viscus. If a flat abdominal radiograph does not reveal evidence of free air, a CT scan should be obtained. If perforation is confirmed, the patient, despite the advanced liver disease, needs surgical exploration.

11. A 47-year-old man is referred for evaluation of ascites believed to be caused by chronic liver disease. There is no history of excessive

alcohol use, risk factors for hepatitis B and C, or other gastrointestinal symptoms. There is a remote history of tuberculosis 20 years earlier managed appropriately. Previous physical examinations have

been said to be unremarkable except for ascites. Repeated physical examination reveals normal vital signs except for pulsus paradoxus of 20 mm Hg. Inspiratory distention of the cervical neck veins is noticed. A third heart sound is heard to the left of the sternal border. The lungs are clear to auscultation. Abdominal examination reveals obvious ascites with a fluid wave. There is no peripheral edema. Ultrasound examination confirms the presence of ascites, reveals a liver that spans 15 cm, and a normal-sized spleen. The portal and hepatic veins are patent.

Key: The presence of pulsus paradoxus and a Kussmaul's sign (inspiratory distention of the cervical neck veins) point to a diagnosis of constrictive pericarditis manifesting as ascites and masquerading as chronic liver disease. Results of an echocardiogram confirmed the diagnosis.

12. A 47-year-old woman is referred for evaluation of hepatosplenomegaly. The patient has no risk factors for chronic liver disease. These include absence of alcohol intake, risk factors for hepatitis B and C, excessive use of vitamins or herbal teas. Urinalysis, however, reveals the presence of hematuria. CT scan shows a lesion in the right kidney consistent with renal cell carcinoma. What is the cause of the patient's hepatosplenomegaly?

    Key: Although one would be tempted to consider metastatic liver disease to be the most likely explanation for the hepatosplenomegaly, a rare syndrome termed *Stauffer's syndrome*, is a form of nephrogenic hepatosplenomegaly. Hypernephroma, in some ill-defined way, may elaborate hepatotropic growth factors that cause enlargement of the liver and spleen. Such patients do not usually have evidence of metastatic liver disease, and with nephrectomy, the liver may actually regress in size.

13. A 57-year-old woman is referred for evaluation of ascites. She initially visited her gynecologist, who suspected that she might have ovarian carcinoma. However, a pelvic examination and pelvic and abdominal CT scans did not reveal any evidence of tumor. There is no history to explain the development of ascites except that the patient has been drinking enormous quantities of herbal tea, ingesting 18 to 24 cups/day for the last 2 years.

    Key: An ultrasound examination with determination of portal and hepatic venous flow velocity revealed findings consistent with hepatic venous occlusion. Tumors (hepatocellular carcinoma, pancreatic carcinoma, hypernephroma, and gastric carcinoma) are

infrequent causes of Budd-Chiari syndrome. The most common cause is an obvious or incipient myeloproliferative syndrome. Herbal teas may contain alkaloids that can cause both intrahepatic and extrahepatic veno-occlusive disease.

14. A 62-year-old woman has been to the hospital because of pruritus and is found to have jaundice. Except for scleral icterus, the findings of the physical examination are unremarkable. The liver span measures 12 cm at percussion. Liver tests reveal the serum bilirubin level to be 8.0 mg/dL; AST, 400 U/L; ALT, 420 U/L; serum alkaline phosphatase, 500 U/L; normal serum prothrombin time; and normal serum albumin and globulin levels. Results of serum albumin, globulin, and antinuclear antibody tests are all either negative or within normal limits. An ultrasound examination shows a normal liver, gallbladder, and pancreas.

    Key: A detailed history interview reveals that this woman took amoxicillin–clavulanic acid at a dosage of 2.0 g/day for 2 weeks, but this medication was discontinued 3 weeks earlier. Amoxicillin–clavulanic acid has been frequently reported to cause a cholestatic hepatitis. Jaundice and abnormal results of liver tests can develop 1 to 4 weeks after discontinuation of treatment. Histologic examination of the liver usually shows evidence of centrilobular (zone 3) or panlobular cholestasis, which, on rare occasions, is associated with a granulomatous hepatitis. The prognosis is excellent.

15. A 43-year-old man with a history of excessive alcohol ingestion (6 to 12 bottles of beer per day for many years) is admitted to the hospital with right upper quadrant pain, chills, fever, dark urine, and pruritus. Physical examination reveals right upper quadrant tenderness, the liver measuring 14 cm with a round, smooth, tender edge palpable 3 cm below the right costal margin. Liver tests reveal serum bilirubin, 8.0 mg/dL; AST, 220 U/L; ALT, 400 U/L; serum albumin, 3.8 g/dL; serum globulins, 3.2 g/dL; serum alkaline phosphatase, 400 U/L; mean corpuscular volume, 94 μm^3; white blood cell count, 14,000/μL with left shift; prothrombin time, 13 seconds (INR, 1.2).

    Key: In patients with alcoholic hepatitis, the AST/ALT ratio is almost invariably 2:1 or greater. In this case, although the patient has consumed excessive amounts of alcohol, the disproportionately higher ALT/AST ratio suggests a cause of jaundice other than alcoholic hepatitis. An ultrasound examination showed cholelithiasis and a dilated common bile duct. This patient, with a history of alcoholism, had biliary tract disease. A clue to the correct diagnosis was the unanticipated reversal of the AST/ALT ratio.

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Editors: **Schiff, Eugene R.; Sorrell, Michael F.; Maddrey, Willis C.**
Title: ***Schiff's Diseases of the Liver, 10th Edition***

Chapter 2
Laboratory Tests

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Marshall M. Kaplan

Key Concepts

- Laboratory tests are an effective method for screening for the presence of hepatic dysfunction, directing further diagnostic evaluation of identified abnormalities, assessing the severity of disease, following the course of liver disease, and evaluating the response to treatment.
- Elevations in serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are nonspecific indicators of hepatocellular damage. The exception is an AST/ALT ratio greater than 2, which suggests alcoholic liver disease.
- Elevation of serum level of alkaline phosphatase in patients with hepatic dysfunction is caused by the regurgitation of alkaline phosphatase from damaged hepatocytes into the serum.
- The rate-limiting step in hepatic bilirubin production is the excretion of conjugated bilirubin into canalicular bile. This explains why patients with significant hepatocellular dysfunction have a predominantly conjugated fraction in hyperbilirubinemia.
- Significant prolongation of the prothrombin time that is unresponsive to vitamin K infusions suggests a poor prognosis in patients with fulminant liver disease.
- The roles of indocyanine green clearance, serum bile salts, aminopyrine breath test, galactose clearance, caffeine clearance, and lidocaine metabolite formation in the evaluation of patients with hepatic dysfunction are limited. These tests are neither widely used nor available.

Laboratory tests, often called *liver function tests* (LFTs), are useful in the evaluation and treatment of patients with hepatic dysfunction. First, they are a sensitive, noninvasive method of screening for the presence of liver dysfunction. This is particularly important for anicteric patients who may have unsuspected disorders, such as viral hepatitis, chronic active hepatitis, cirrhosis, or partial bile duct obstruction. Second, once the presence of hepatic dysfunction is recognized, the pattern of laboratory test abnormalities may allow clinicians to recognize the general type of liver disorder. For example, laboratory tests usually allow clinicians to differentiate hepatocellular disorders, such as viral hepatitis, from cholestatic syndromes, such as primary biliary cirrhosis and bile duct obstruction. Third, laboratory tests allow clinicians to assess the severity of liver dysfunction and occasionally allow them to predict the outcome early in the course of disease. Finally, LFTs allow the physician to follow the course of liver disease, to accurately evaluate the response to treatment, and to adjust treatment when necessary.

Although they are indispensable in the treatment of patients with liver disease, laboratory tests have certain limitations. First, they lack sensitivity. Normal results may be obtained for patients with serious liver disorders, such as cirrhosis or hepatocellular carcinoma. Second, these tests are not specific for liver dysfunction. For example, the serum albumin level may be decreased in patients with chronic inflammatory conditions, and aminotransferase levels may be elevated in patients with musculoskeletal or cardiac diseases. Finally, results of LFTs seldom lead to a specific diagnosis; rather, they suggest a general category of liver disorder. They do not help

differentiate viral hepatitis from drug-induced hepatitis or intrahepatic cholestasis from extrahepatic bile duct obstruction.

No one LFT enables the clinician to accurately assess the total functional capacity of the liver. The liver performs thousands of biochemical functions, most of which cannot be measured easily with blood tests. Laboratory tests measure only a limited number of these functions. Many tests, such as aminotransferase and alkaline phosphatase determinations, do not measure liver function at all. Rather, they show liver cell damage or interference with bile flow. To increase both the sensitivity and the specificity of laboratory tests in the detection of liver disease, it is essential to use them as a battery and to repeat them over time. When more than one of these tests provide abnormal findings, or the findings are persistently abnormal on serial determinations, the probability of liver disease is high. When all the test results are normal, the likelihood of liver disease is low.

In the evaluation of patients for liver disorders, it is helpful to group these tests into general categories. We have found the following classification most useful:

Tests of the capacity of the liver to transport organic anions and metabolize drugs: Included in this group are serum bilirubin, sulfobromophthalein sodium (bromsulphalein [BSP]), indocyanine green (ICG), serum bile acids, serum caffeine, serum lidocaine metabolites, and breath tests. Each of these tests measures the ability of the liver to clear endogenous or exogenous substances from the circulation.

Tests to detect injury to hepatocytes: These include all of the enzyme tests, of which the aminotransferases and alkaline phosphatase are the most commonly ordered and the most useful.

Tests of the biosynthetic capacity of the liver: Included in this group are serum albumin, ceruloplasmin, ferritin, α_1-antitrypsin, lipoproteins, and blood-clotting factors. These substances are synthesized in the liver for transport into the circulation.

Tests to detect fibrosis in the liver: These include serum hyaluronate, type IV collagen, procollagen III, laminin, and multiparameter tests including FibroTest.

Tests to chronic inflammation or altered immunoregulation: These include the immunoglobulins (Igs), and specific autoantibodies. These are not truly LFTs, because most of these substances are proteins made by B lymphocytes, not by hepatocytes. Some of these tests, however, are quite specific for certain liver diseases.

This chapter discusses the tests we consider most useful in evaluating liver diseases. Other tests that are of less value but are still used are discussed briefly. Whenever possible, the pathophysiologic basis of each test is reviewed together with the sensitivity and specificity of the test in assessing of the nature and severity of liver dysfunction.

Tests of Capacity of Liver to Transport Organic Anions and to Metabolize Drugs

Bilirubin

See Chapter 8.

Bilirubin metabolism

Bilirubin, a tetrapyrrole pigment, is a breakdown product of ferroprotoporphyrin IX (heme), an integral part of heme-containing proteins. Approximately 70% to 80% of the 250 to 300 mg of bilirubin produced each day is derived from the breakdown of hemoglobin in senescent red blood cells (1). The remainder comes from prematurely destroyed erythroid cells in bone marrow and from the turnover of hemoproteins in tissues throughout the body (2). The liver is the main source of the latter because of its high concentration of hemoproteins with relatively high turnover rates, such as cytochrome P-450. The initial steps leading to the formation of bilirubin occur in reticuloendothelial cells, primarily in the spleen and liver. The first reaction, catalyzed by the microsomal enzyme heme oxygenase, oxidatively cleaves the α bridge of the porphyrin group, opens the heme ring, and produces equimolar amounts of biliverdin and carbon monoxide (3) (Fig. 2.1). The second reaction, catalyzed by the cytosolic enzyme biliverdin reductase, reduces the central methylene bridge of biliverdin, converting it to bilirubin (4). Bilirubin formed in the reticuloendothelial cells is lipid-soluble and almost insoluble in water. To be transported in blood, it must be solubilized. This is accomplished by reversible, noncovalent binding to albumin. Bilirubin is then transported to the liver, where it, but not the albumin, is taken up by hepatocytes in a process that involves carrier-mediated membrane transport (5) (Fig. 2.2). Although several potential transporters have been identified, none has yet been cloned. In the hepatocyte, bilirubin is coupled to glutathione-*S*-transferases (formerly called ligandins). Bilirubin is then solubilized by means of conjugation to glucuronic acid, and bilirubin monoglucuronide and diglucuronide are formed, both of which are called *direct-acting bilirubins.* The conjugation of glucuronic acid to bilirubin is catalyzed by an enzyme system in the endoplasmic reticulum of the hepatocyte that transfers glucuronic

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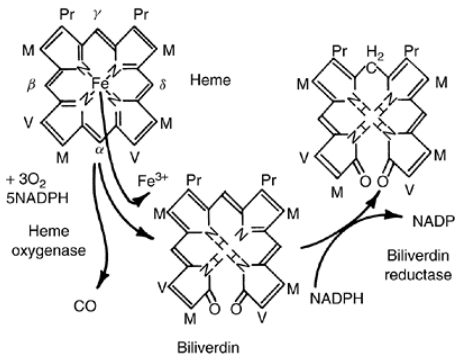
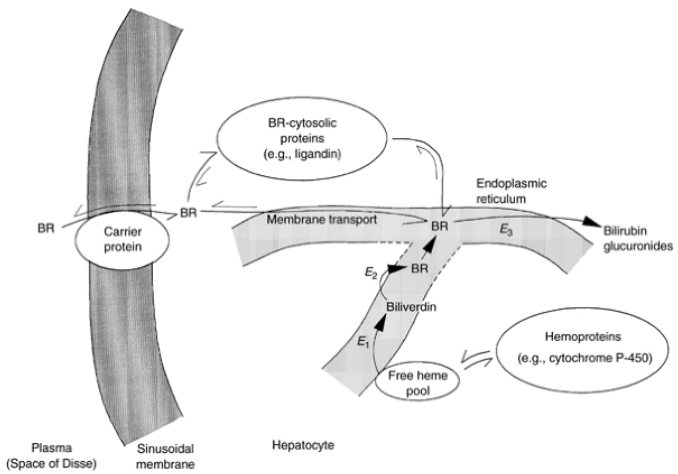
acid from uridine diphosphate (UDP)-glucuronic acid to the acyl groups of the propionic acid side chains of bilirubin (Fig. 2.3). The bilirubin conjugates are then actively transported from the hepatocyte into canalicular bile by an adenosine triphosphate–dependent transport process that is the rate-limiting step in hepatic bilirubin excretion. This process is mediated by a protein in the bile canalicular membrane called *multidrug resistance–associated protein 2.* The conjugated bilirubins drain from the bile duct into the duodenum and are carried distally through the intestine. In the distal ileum and colon, the conjugated bilirubins are hydrolyzed to unconjugated bilirubin by bacterial β glucuronidases. The unconjugated bilirubin is reduced by normal intestinal bacteria to form a group of colorless tetrapyrroles called *urobilinogens.* Approximately 80% to 90% of these products are excreted in feces, either unchanged or oxidized to orange derivatives called *urobilins.* The remaining 10% to 20% of the urobilinogens are passively absorbed, enter the portal venous blood, and are reexcreted by the liver. A small fraction, usually less than 3 mg/dL, escapes hepatic uptake, filters across the renal glomerulus, and is excreted in urine. The renal excretion of urobilinogen is complicated, partly because urobilinogen is a weak acid that passively diffuses across the renal tubule when in its undissociated form. The appearance of urobilinogen in urine depends on many factors, including urine pH and the rate of urine flow.

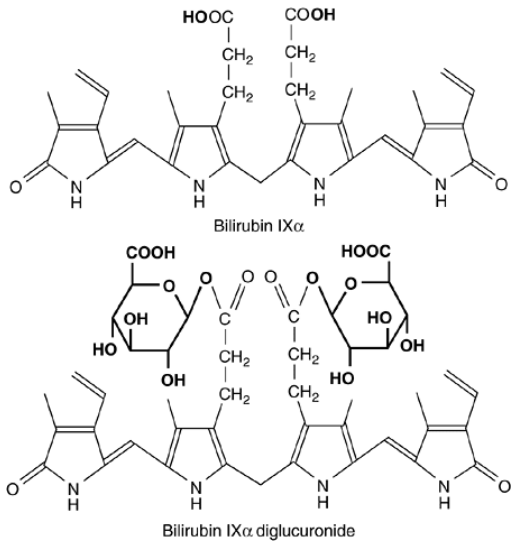


• **Figure 2.1** Formation of bilirubin from heme by the sequential actions of the enzymes heme oxygenase and biliverdin reductase. *M*, methyl; *V*, vinyl; *Pr*, propionic acid side chains; CO, carbon monoxide; NADPH, reduced form of nicotine adenine dinucleotide; NADP, nicotine adenine dinucleotide phosphate. (Adapted from Ostrow JD, et al. *Unit 1: Hepatic excretory function: Undergraduate teaching project*. American Gastroenterological Association, Milner-Fenwick Slides Co., with permission.)



• **Figure 2.2** Scheme of bilirubin uptake, hepatocellular transport, and metabolism. Exogenous bilirubin is taken up by the hepatocyte at the sinusoidal membrane by an unidentified carrier protein (as depicted) or by transmembrane "flip-flop." The

bilirubin is delivered to the endoplasmic reticulum by means of membrane–membrane transfer, with cytosolic binding proteins serving as a potential means to preclude the diffusion of bilirubin back to the plasma. Heme generated within the liver from endogenous hemoproteins also gives rise to bilirubin. Glucuronidation occurs primarily within the endoplasmic reticulum. *BR*, bilirubin; E_1, heme oxygenase; E_2, biliverdin reductase; E_3, bilirubin uridine diphosphate glucuronosyltransferase 1. (From Crawford JM, Hauser SC, Gollan JL. Formation, hepatic metabolism, and transport of bile pigments: a status report. *Semin Liver Dis* 1988;8:105, with permission.)



• **Figure 2.3** Structure of bilirubin IXα and bilirubin IXα diglucuronide. In the latter, the glucuronides prevent intramolecular hydrogen bonding between the propionic acid groups and the pyrrole ring nitrogens. (From Schmid R. Bilirubin metabolism: State of the art. *Gastroenterology* 1978;74:1307, with permission.)

Measurement of serum bilirubin

The terms *direct-* and *indirect-reacting bilirubin* are based on the original van den Bergh method of measuring unconjugated bilirubin (6). This method is still used in some clinical chemistry laboratories to determine the serum bilirubin level. In this assay, bilirubin reacts with diazotized sulfanilic acid and splits into two relatively stable dipyrryl azopigments that absorb maximally at 540 nm. The direct fraction is that which reacts with diazotized sulfanilic acid in 1 minute in the absence of alcohol (6). This fraction provides an approximate determination of the amount of conjugated bilirubin in serum. The total serum bilirubin level is the amount that reacts in 30 minutes after the addition of alcohol. The indirect fraction is the difference between the total and the direct bilirubin level, and provides an estimate of the amount of unconjugated bilirubin in serum. With the van den Bergh method, the normal serum bilirubin concentration is usually less than 1 mg/dL (18 mmol/L). As much as 30%, or 0.3 mg/dL (5.1 mmol/L) of the total is direct-reacting bilirubin (6). Total serum bilirubin concentrations are between 0.2 and 0.9 mg/dL (2.0 to 15.4 mmol/L) in 95% of a healthy

population, and less than 1 mg/dL (18 mmol/L) in 99% (6). Some differences in the properties of unconjugated and conjugated bilirubin are listed in Table 2.1.

Table 2.1. Differences Between Unconjugated and Conjugated Bile Pigments

Property	Unconjugated bilirubin	Conjugated bilirubin
Van den Bergh reaction	Indirect (+ alcohol)	Direct
Water soluble	-	+
Fat soluble	+	-
Attachment to plasma albumin	+	+
Presence in icteric urine	-	+
Presence in bile	-[a]	+
Affinity for brain tissue	+	-
Association with hemolytic jaundice	++	±

Association with obstructive and hepatocellular jaundice	+	+++

[a]A small quantity of unconjugated bilirubin may be present in the common duct bile. There is a relative increase in unconjugated bilirubin in bile in conditions associated with severe unconjugated hyperbilirubinemia, such as Crigler-Najjar syndrome (7).
+, present or positive; -, absent or negative; ±, present or absent; ++, strong; +++, very strong.

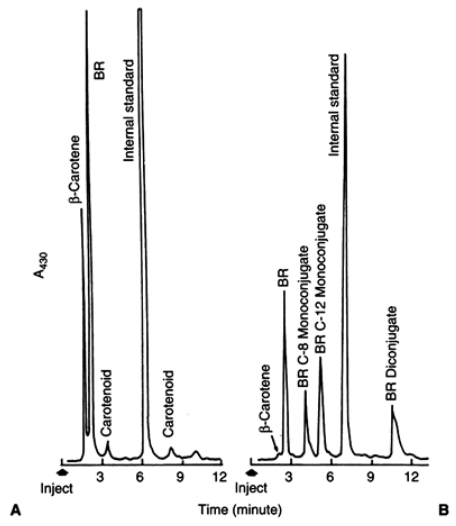
Advances in methodology have shown that the diazo method does not accurately reflect the values of the indirect- and direct-reacting fractions of bilirubin, particularly at low total serum bilirubin concentrations (8). Although these more accurate methods provided information that resolved many of the enigmas of bilirubin metabolism, they are less convenient to perform. In most clinical situations, they do not provide enough clinical advantages to replace the older, more familiar diazo methods. One more precise method involves alkaline methanolysis of bilirubin, followed by chloroform extraction of the bilirubin methyl esters, separation of these esters with high-performance liquid chromatography (HPLC), and spectrophotometric determination at 430 nm (8). Other HPLC methods do not require alkaline methanolysis, although globulins and other high-molecular-weight proteins must be precipitated from serum before chromatography. Assays based on dry reagent chemistry have also been reviewed. One method used in many clinical chemistry laboratories is based on photographic film technology, and uses Ektachem dry chemistry slides. The method can be automated and appears able to produce accurate measurements of conjugated and unconjugated bilirubin to demonstrate the presence of a bilirubin fraction called *bilirubin δ.* Bilirubin δ is conjugated bilirubin that is tightly linked to albumin through covalent bonding (9).

These new techniques considerably add to our understanding of bilirubin metabolism. First, they show that almost 100% of the serum bilirubin in healthy individuals, or those with Gilbert's syndrome, is unconjugated (Fig. 2.4), and less than 3% is monoconjugated bilirubin. Second, in patients with jaundice and hepatobiliary disease, the total serum bilirubin concentration measured with these newer, more accurate methods is lower than the values found with diazo methods (8). This suggests that diazo-positive compounds distinct from bilirubin exist in the serum of patients with hepatobiliary disease. Third, the results of these studies indicate that in patients with jaundice and hepatobiliary disease, monoglucuronides of bilirubin predominate over the diglucuronides (Fig. 2.4). Fourth, part of the direct-reacting bilirubin fraction includes conjugated bilirubin that is covalently linked to albumin (9) (Fig. 2.5). This albumin-linked bilirubin fraction represents an important fraction of total serum bilirubin in patients with cholestasis and hepatobiliary disorders (9) (Fig. 2.6). Albumin-bound bilirubin is formed in serum when hepatic excretion of bilirubin glucuronides is impaired, and the glucuronides are present in serum in increasing amounts. Because of its tight binding to albumin, the clearance rate of albumin-bound bilirubin from serum approximates the half-life of albumin—12 to 14 days—rather than the short half-life of bilirubin, approximately 4 hours. The prolonged half-life of albumin-bound bilirubin explains two previously unexplained enigmas in the care of jaundiced patients with liver disease: (a) That some patients with conjugated hyperbilirubinemia do not have bilirubinuria during the recovery phase of their disease and (b) that the elevated serum bilirubin level declines more slowly than expected in some patients who otherwise appear to be recovering satisfactorily. Late in the recovery phase of hepatobiliary disorders, all the conjugated bilirubin may be in the albumin-linked form. It is therefore not filtered by the renal glomerulus and does not appear in urine, although the serum bilirubin concentration is high. The value of conjugated bilirubin in serum decreases slowly because of the long half-life of albumin. The slow decline is unrelated to the actual hepatic status.

Diagnostic value of serum bilirubin

The bilirubin normally present in serum represents a balance between the input from production and the hepatic removal of the pigment. Hyperbilirubinemia may therefore result from (a) overproduction of bilirubin, (b) impaired uptake, conjugation, or excretion of bilirubin, or (c) regurgitation of unconjugated or conjugated bilirubin from damaged hepatocytes or

bile ducts. One may anticipate that an increase in unconjugated bilirubin in serum results from overproduction or from impairment of uptake or conjugation, whereas an increase in the conjugated moiety is caused by decreased excretion or backward leakage of the pigment.



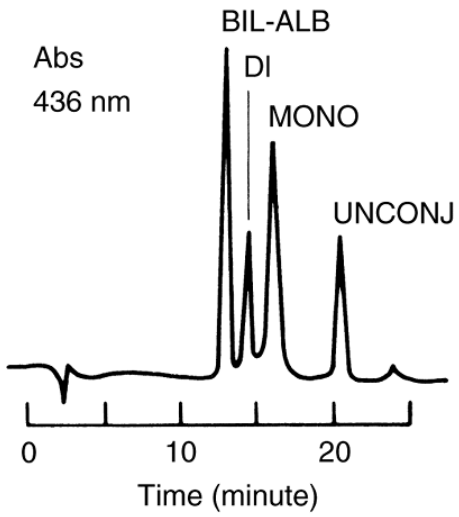
• **Figure 2.4** Chromatograms of bilirubin in the plasma of a healthy adult **(A)** and a patient with obstructive jaundice. **B:** Bilirubin glucuronides are not detected in the healthy adult. In the patient with obstructive jaundice, there is a mixture of unconjugated bilirubin, bilirubin monoglucuronides, and bilirubin diglucuronide. Bilirubin conjugates in plasma were converted to their methyl ester derivatives and then extracted and separated by means of high-pressure liquid chromatography. Xanthobiliburic acid was used

as an internal standard. *BR*, unconjugated bilirubin. (From Blanckaert N, Schmid R. Physiology and pathophysiology of bilirubin metabolism. In: Zakim D, Boyer TA, eds. *Hepatology: A Textbook of Liver Disease*, 2nd ed. Philadelphia, PA: WB Saunders, 1989:262, with permission.)

Total serum bilirubin level is not a sensitive indicator of hepatic dysfunction and may not accurately reflect the degree of liver damage. Hyperbilirubinemia may not be detected in instances of moderate to severe hepatic parenchymal damage or a partially or briefly obstructed common bile duct. This lack of sensitivity is partly explained by observations obtained in healthy persons given infusions of unconjugated bilirubin and in patients with uncomplicated hemolysis. These observations suggest that the capacity of the human liver to remove bilirubin from serum before hyperbilirubinemia occurs is at least twofold greater than the daily pigment load (250 to 300 mg [4,275 to 5,130 mmol]) normally presented to this organ. This capacity may be even higher, according to the maximal rate of excretion of bilirubin into bile—approximately 55.2 mg/kg/day (10)—and the average amount of bilirubin formed from the destruction of senescent red blood cells, 3.9 mg/kg/day. In the steady state, the serum bilirubin concentration usually reflects the intensity of jaundice and the increase in total-body bile pigment. The serum bilirubin concentration may occasionally decrease transiently with the presence in serum of substances such as salicylates, sulfonamides, or free fatty acids, which displace bilirubin from its attachment to plasma albumin and enhance the transfer of the pigment into tissues (11). Conversely, an increase in serum albumin concentration may induce a temporary shift of bilirubin from tissue sites into the circulation.

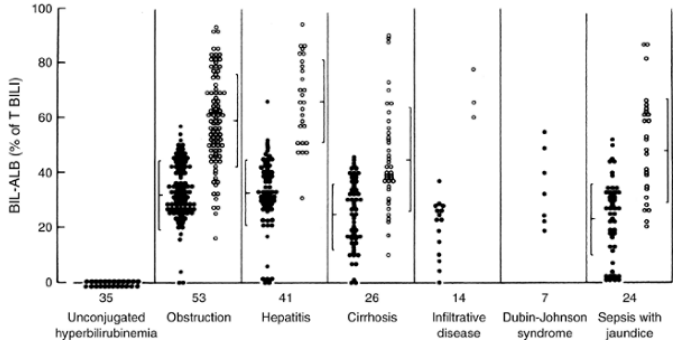
Total serum bilirubin concentration is seldom of value in specifying the cause of jaundice in individual patients because values among the various types of jaundice overlap considerably. On the average, uncomplicated hemolysis seldom causes a serum bilirubin value in excess of 5 mg/dL (85.5 mmol/L), and parenchymal liver disease or incomplete extrahepatic

obstruction due to biliary calculi gives lower serum bilirubin values than those that occur with malignant obstruction of the common bile duct.



▪ **Figure 2.5** Chromatogram of serum bilirubin from a patient with obstructive jaundice shows the presence of a bilirubin fraction bound to albumin (BIL-ALB). Serum bilirubin was separated by means of reversed-phase high-performance liquid chromatography. DI, bilirubin diglucuronide; MONO, bilirubin monoglucuronide; UNCONJ, conjugated bilirubin. (From Weiss JS, Gautam A, Lauff JJ, et al. The clinical importance of a protein-bound fraction of serum bilirubin in patients with hyperbilirubinemia. *N Engl J Med* 1983;309:147, with permission.)

Few controlled studies have critically assessed the prognostic value of magnitude and duration of hyperbilirubinemia in liver disease. In general, the higher the serum bilirubin concentration in viral hepatitis, the greater the histologic evidence of hepatocellular damage and the longer the course of disease. Nevertheless, patients may die of fulminant hepatitis with only a modest elevation in serum bilirubin level. The presence of concomitant hemolysis with overproduction of bilirubin and diminished glomerular filtration rate causing decreased excretion of the pigment may also confuse the issue by causing higher serum bilirubin values than would be expected for any degree of hepatocellular damage present. In acute alcoholic hepatitis, hyperbilirubinemia in excess of 5 mg/dL (85.5 mmol/L) is one of the findings that connotes a poor prognosis (12).



• **Figure 2.6** Albumin-bound bilirubin (BIL-ALB) as a percentage of total bilirubin (T BILI). The number of subjects is indicated for each diagnostic category. Each *solid circle* represents a serum sample from a patient with a clinically deteriorating condition and increasing total bilirubin level. Each *open circle* represents a sample from a patient with clinical improvement and decreasing total bilirubin level. The *bars* indicate means ±1 SD. Serial measurements were not obtained for subjects with unconjugated hyperbilirubinemia and Dubin-Johnson syndrome. (From Weiss JS, Gautam A, Lauff JJ, et al. The clinical importance of a protein-bound fraction of serum bilirubin in patients with hyperbilirubinemia. *N Engl J Med* 1983;309:147, with permission.)

The major value of fractionating total serum bilirubin into unconjugated and direct-reacting moieties is in the detection of states characterized by unconjugated hyperbilirubinemia (Table 2.2). Such a diagnosis appears warranted when the serum level of indirect-reacting bilirubin is in excess of 1.2 mg/dL (20.5 mmol/L) and the direct-reacting fraction constitutes less than 20% of the total serum bilirubin value. Unfortunately, when the total serum bilirubin concentration is minimally elevated, it may be difficult to differentiate the nature of the bilirubin elevation. The difficulty is caused by the inaccuracy of the diazo methods in differentiating conjugated from unconjugated bilirubin at low total serum bilirubin concentrations. This is one of the instances in which the newer, more precise methods of bilirubin determination may provide clinically useful information that is not attainable with the older diazo methods. This is particularly true in the detection of

early or mild liver injury. Total bilirubin concentration may initially be normal in some patients with cirrhosis, hepatitis, congestive heart failure, and other disorders. An increase in the direct fraction above 0.3 mg/dL (5.1 mmol/L) should alert one to the possibility of mild liver injury. If the newer, more accurate techniques are used, conjugated bilirubin concentrations greater than 0.1 mg/dL (1.7 mmol/L) should be accurate in the detection of early liver injury, because bilirubin glucuronides are normally undetectable in serum, except in hepatobiliary disorders (8).

Table 2.2. Causes of Unconjugated Hyperbilirubinemia

Cause	Mechanism
Hemolytic disorders	
Inherited:	
Spherocytosis, elliptocytosis, G-6-phosphate dehydrogenase deficiency, sickle cell anemia	Overproduction of bilirubin
Acquired:	
Microangiopathic hemolytic anemias, paroxysmal nocturnal hemoglobinuria, immune hemolysis	Overproduction of bilirubin
Ineffective erythropoiesis	
Cobalamin, folate and severe iron deficiencies, thalassemia	Overproduction of bilirubin
Drugs	
Rifampicin, probenecid	Impaired hepatic uptake
Inherited conditions	
Gilbert's syndrome	Impaired conjugation
Crigler-Najjar, types I and II	Impaired conjugation
Neonates	
Neonatal (physiologic)	Overproduction of bilirubin, impaired conjugation, increased intestinal absorption
Breast milk	Impaired conjugation and increased intestinal absorption
Other	
Hematoma	Overproduction of bilirubin

Measurement and fractionation of serum bilirubin concentration in patients with jaundice does not allow clinicians to differentiate accurately between parenchymal (hepatocellular) and cholestatic (obstructive) jaundice. The accurate HPLC methods for measuring serum bilirubin show that the levels of both unconjugated and conjugated bilirubins are increased in hepatobiliary disease. No consistent pattern of elevation of these fractions differentiates hepatocellular from cholestatic liver disease (8). Levels of both monoglucuronide and diglucuronide are elevated, the monoglucuronides predominating.

Urinary bilirubin

The presence of bilirubin in the urine indicates the presence of hepatobiliary disease. Unconjugated bilirubin is tightly bound to albumin, not filtered by the glomerulus, and not present in urine. Consequently, only conjugated bilirubin is found in urine. This occurs only when conjugated bilirubin is in the serum, that is, when there is hepatobiliary disease. The new, more precise methods for measuring serum bilirubin indicate that 100% of the serum bilirubin in healthy persons and those with Gilbert's syndrome is unconjugated bilirubin. Measurable amounts of conjugated bilirubin in serum are found only in hepatobiliary disease. Because the renal threshold for conjugated bilirubin is low and the laboratory methods used can detect bilirubin concentrations as low as 0.05 mg/dL (0.9 mmol/L) of urine, conjugated bilirubin may be found in urine when the total serum bilirubin value is normal and the patient does not have clinical jaundice. This can occur early in the course of viral hepatitis or other hepatobiliary diseases when conjugated bilirubin first appears in the serum. Conversely, the urine can become free of bilirubin long before the level of conjugated serum bilirubin falls to normal in patients recovering from hepatobiliary diseases (9). When this occurs, all the conjugate bilirubin is in the albumin-bound form and is not filtered by the glomerulus.

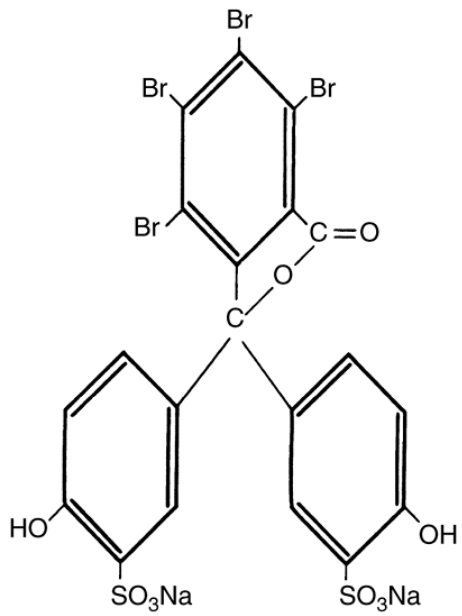
Dye Tests

Because it has long been recognized that serum bilirubin is an insensitive test of hepatic dysfunction and that at low serum levels measurement of total and direct bilirubin lacks biochemical precision, an effort was made to develop other tests of hepatic excretory capacity that were more sensitive and specific. The goal was to develop tests that could be used for more critical and specific evaluations of the excretory or detoxification capacity of the liver. Although all these tests are clearly more sensitive than the serum bilirubin test, they have limited value because of their nonspecificity; that is, the extent of the abnormality is comparable in all types of hepatobiliary disorders. These tests include dye, breath, caffeine clearance, and serum bile acid tests.

Sulfobromophthalein sodium

BSP (Fig. 2.7) was introduced in 1924 and used as a sensitive indicator of liver dysfunction until the 1970s, when its use greatly decreased. BSP is seldom used now and has been withdrawn from commercial

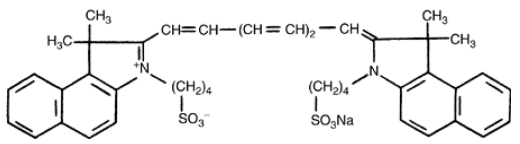
markets because of a decline in sales caused by fear of potentially fatal anaphylactic reactions after intravenous administration. The following discussion is included more for historical interest than for practical use by hepatologists and clinical investigators.



• **Figure 2.7** Structural formula for sulfobromophthalein sodium (sodium phenoltetrabromophthalein disulfonate).

BSP is soluble in water but binds rapidly to albumin and α_1-lipoprotein when it is injected intravenously. BSP or the BSP-albumin complex binds to the hepatocyte plasma membrane, and BSP is avidly taken up by the hepatocyte, where it binds to glutathione-*S*-transferases. Hepatic uptake of BSP appears saturable and there is competition for uptake with bilirubin and ICG. BSP has a first-pass clearance by the liver of 50% to 80%, and its removal from the circulation is closely related to hepatic blood flow. Much of the BSP within hepatocytes is conjugated to glutathione in a thioether linkage. The reaction is catalyzed by glutathione-*S*-transferases, the same proteins that bind BSP and bilirubin within hepatocytes. Both conjugated and free BSP are then excreted across canalicular membranes into the biliary tract. Conjugation is not required for uptake or biliary excretion of the dye.

Prolonged infusions of BSP have been used to quantify the excretory capacity of the liver by means of measurement of the hepatic uptake, storage, and maximal biliary excretion rate of BSP. Similar types of data have been generated by means of simple determination of the plasma disappearance after a standard intravenous injection of BSP. The standard test involves the intravenous injection of a fixed dose of BSP—usually 5 mg/kg—followed by sampling of blood from the contralateral arm at various times after injection. Serum is separated and alkalinized, and the BSP is read at 580 nm in a spectrophotometer. The purpose is to determine the percentage of dye remaining in plasma at a given time after injection. Normal values at 30 and 45 minutes are 10% and 5%, respectively, although these values may exclude up to 30% of healthy persons.



• **Figure 2.8** Structural formula for indocyanine green

Because the BSP retention test depends on so many factors—including hepatic blood flow, hepatic mass, functional capacity of hepatocytes, and bile flow—the test result is abnormal in all types of liver disease. As would be expected, it is also nonspecific. In addition, results are affected by fever and the concomitant use of drugs that either displace BSP from its plasma-binding proteins or inhibit its uptake or excretion by the liver. There is one clinical situation in which the BSP test is useful: The diagnosis of Dubin-Johnson syndrome and its differentiation from Rotor's syndrome. In Dubin-Johnson syndrome, an initial rapid decrease in plasma BSP concentration is followed by a secondary increase 45 to 90 minutes later (13). This reaction is caused by the regurgitation of conjugated BSP. In Rotor's syndrome, the clearance of BSP from serum is slower and no secondary increase occurs.

Indocyanine green

ICG (Fig. 2.8) is another dye used to evaluate liver function (14). ICG is nontoxic, has a higher hepatic extraction ratio than BSP (70% to 96%), and is bound to albumin and α_1-lipoprotein more avidly than BSP. Ninety-seven percent of an administered dose is excreted unchanged in bile, and the liver appears to be the only site of ICG clearance for humans. ICG and BSP appear to share sinusoidal and canalicular transport systems, but ICG has no enterohepatic circulation. ICG can be measured directly with spectrophotometry.

ICG disappearance curves can theoretically be measured without repeated blood samplings by means of dichromatic earlobe densitometry (14) or with a fingertip optical sensor (15). Results with both of these noninvasive methods appear to correlate well with those of blood sampling (15,16,17,18).

ICG is used primarily to estimate hepatic blood flow. If ICG is infused intravenously at a rate well below the capacity of the liver to clear it, a steady state is reached within 1 hour. At that time, the rate of hepatic clearance is equal to the infusion rate. Because this is known and the ICG concentration in peripheral venous blood is easily measured, hepatic blood flow can be estimated by use of the Fick equation, as follows:

$$F = \frac{R}{(ICG)a - (ICG)hv}$$

where F is the estimated hepatic blood flow; R is the hepatic removal rate of ICG, which equals the infusion rate at steady state; (ICG)a is the concentration of ICG in the hepatic artery and portal venous blood; and (ICG)hv is the concentration of ICG in hepatic venous blood.

(ICG)a is the same as the concentration of ICG in peripheral venous blood. Measurement of (ICG)hv would require catheterization of the hepatic vein and is impractical in most clinical settings. Because first-pass extraction of ICG by the liver is high (70% to 96%), the clearance rate is assumed to be 100%, and (ICG)hv is set at zero accordingly. This method can be performed at the bedside; however, the result is an underestimate of hepatic blood flow because (ICG)hv is always more than zero. This method provides a good approximation of blood flow in healthy adults (19), but not in patients with cirrhosis, because of a marked decrease in ICG extraction (20).

ICG clearance is not as sensitive an indicator of hepatic dysfunction as BSP because at doses of ICG that are safe to administer, the excretory capacity of the liver for ICG is far below maximum. To estimate the maximal ICG capacity, one would have to use four or five doses of ICG at different times and then use Michaelis-Menton–type data analysis. This is time-consuming and impractical, except in a research setting. Those who have used ICG in the evaluation of liver disease recommend single intravenous injections ranging from 0.64 to 6.4 mmol/kg and determination of a single blood level 20 minutes later. Both doses are well below the dose needed to saturate the excretory capacity of the liver for ICG, which is more than 72 mmol/kg. One group has described a new method to determine the maximal removal rate of ICG with a single submaximal dose and continuous measurement of serum ICG concentration with a fingertip optical sensor (21).

Unlike the BSP test, ICG clearance is not affected by fever (14) and is normal in patients with the Dubin-Johnson syndrome and in neonates (14). Studies have looked at the utility of ICG clearance as a predictor of poor outcome due to hepatic dysfunction in patients undergoing cardiac surgery and in patients with septic shock (22,23,24). However, because of the problems discussed earlier, ICG is seldom used in the routine evaluation of patients with liver disease.

Bile Acid Tests

Although sensitive tests to measure serum bile acid concentration (see Chapter 9) have been available for more than 20 years, physicians are still searching for a role for these tests in the evaluation of patients with suspected liver disease. Bile acids are synthesized from cholesterol in hepatocytes, conjugated to glycine or taurine, and then secreted into bile. Approximately 80% to 90% of the bile acids are stored in the gallbladder between meals; the remaining fraction is secreted continuously into the duodenum. This fraction accounts for the bile acids normally present in serum after a long fast, the concentration of which is 5 to 10 mmol/L. Cholic acid conjugates constitute less than 20% of this amount, and dihydroxy bile acids constitute the remainder.

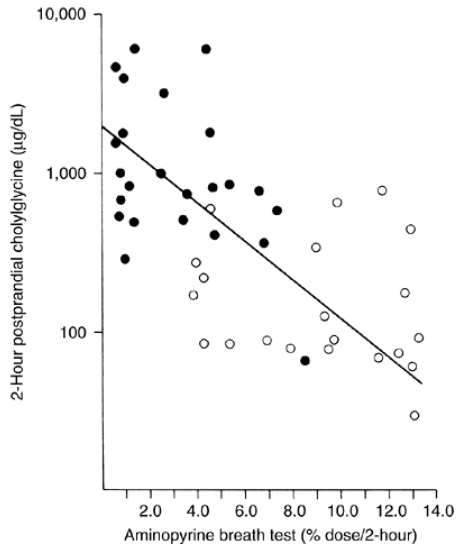
During a meal, the gallbladder contracts and discharges its pool of bile acids into the duodenum. Bile acids move rapidly down the intestinal tract, where some are absorbed throughout the intestine by means of nonionic passive diffusion. Most are actively reabsorbed by carrier-mediated transport in the terminal ileum and carried back to the liver through the portal vein. The liver efficiently extracts bile acids from portal blood; approximately 70% to 80% of dihydroxy bile acids undergo first-pass extraction, whereas 90% of trihydroxy bile acids are extracted. This difference in extraction rates is probably due to the tighter binding of dihydroxy bile acids to albumin. The fractional extraction rates of bile acids are relatively constant in healthy persons. Because a larger quantity of bile acids reach the liver after a meal and the proportion extracted is constant, a larger quantity of bile acids escapes into the circulation postprandially. This produces the normal postprandial increase in serum bile acid concentration, to a level approximately two- to fivefold greater than fasting level. In health, all the serum bile acids are from intestinal input; none comes directly from the liver. Maintenance of normal serum bile acid concentrations depends on hepatic blood flow, hepatic uptake, secretion of bile acids, and intestinal motility. A disease that affects

any of these functions should theoretically affect serum bile acid levels. This has proved to be true in practice. Serum bile acids are sensitive but nonspecific indicators of

hepatic dysfunction. They allow some quantification of functional hepatic reserve.

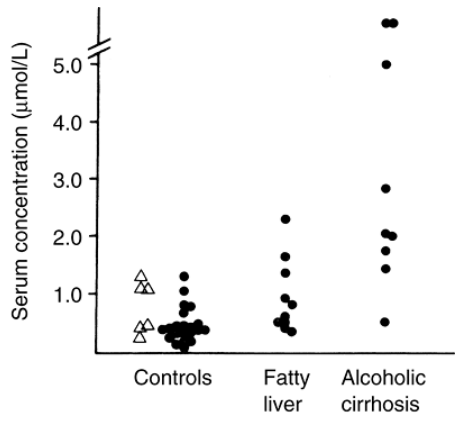
Several ways to accurately measure serum bile acid concentration are available. These include enzymatic assays, in which the bacterial enzyme 3α-hydroxysteroid dehydrogenase is coupled with either fluorometric or bioluminescence techniques; gas–liquid chromatography; radioimmunoassay; and a highly specific assay that combines gas–liquid chromatography and mass spectrometry. Only the enzyme assays and radioimmunoassays are easily adapted to the clinical chemistry laboratory. Although the radioimmunoassays can be automated, a variety of individual radioimmunoassays to detect cholic and chenodeoxycholic acids and their conjugates would have to be used to measure the total amount of bile acids in serum. This is probably not necessary if the purpose of the assay is to detect liver disease.

Serum bile acid tests may produce disproportionately elevated results in certain cholestatic liver diseases. They are useful in the management of primary biliary cirrhosis and primary sclerosing cholangitis. Serum bile acid levels may be increased 25-fold in some patients. The response to treatment with ursodeoxycholic acid can be monitored by means of measurement of the concentrations of the serum bile acids. With adequate dosing, ursodeoxycholic acid eventually constitutes nearly 60% of serum bile acids (25). Serum bile acid levels are elevated in pregnant women and particularly increased in women with pruritus of cholestasis. There appears to be no consistent advantage to the postprandial test compared with the fasting test. As might be expected, the bile acid tests are more sensitive than the serum bilirubin tests in all types of hepatobiliary disorders and correlate moderately well with results of the aminopyrine breath test (Fig. 2.9) (26). Serum bile acid levels are normal in patients with Gilbert's syndrome and Dubin-Johnson syndrome. Serum bile acid tests are as sensitive as aminotransferase measurement in the detection of acute viral hepatitis but less so than aminotransferase measurement in the detection of posttransfusion hepatitis. They are also less sensitive than aminotransferase determination as screening tests for subclinical liver disease. Serum bile acid levels are invariably abnormal in cirrhosis of any cause, and the test is equal to or more sensitive than tests such as the serum albumin measurement or prothrombin time. This finding is not unexpected and is due to the decreased functioning liver cell mass, decreased bile excretion, and portosystemic shunting usually present in chronic liver diseases, all of which affect serum bile acid levels. It has been suggested that the results of serum bile acid tests are predictive of histologic severity (26) and might replace percutaneous liver biopsy; however, investigators have found a poor correlation between serum bile acid levels and histologic severity in patients with chronic hepatitis or alcoholic liver disease (Fig. 2.10) (27,28). There is too much overlap between serum bile acid levels in patients with chronic persistent hepatitis and chronic active hepatitis with bridging necrosis or cirrhosis to obviate liver biopsy. Results of one small study suggested that total serum bile acid levels may be an indication of histologic improvement in patients with chronic hepatitis C who respond to interferon therapy (29).



▪ **Figure 2.9** Correlation between the aminopyrine breath test and the logarithm of 2-hour postprandial serum cholylglycine levels in 44 patients who underwent both tests. *Closed circles* indicate chronic active hepatitis with bridging necrosis or cirrhosis; *open circles* indicate chronic persistent hepatitis and chronic active hepatitis. Cholylglycine levels were determined by means of radioimmunoassay. (From Monroe PS, Baker AL, Schneider JF, et al. The aminopyrine breath test and serum bile acids reflect histologic severity in chronic hepatitis. *Hepatology* 1982;2:317, with permission.)

The attempt to improve the sensitivity and specificity of the serum bile acid tests with intravenous or oral tolerance tests has not proved successful. The ratio of cholic acid to chenodeoxycholic acid has also been considered a means of increasing the sensitivity and specificity of bile salt testing. Unfortunately, a marked overlap exists, both between the healthy control and various hepatic disease states. Preliminary data suggest that a drop in the cholic acid to chenodeoxycholic acid ratio may be helpful in differentiating graft rejection from other causes of hepatic dysfunction in recipients of liver transplants (30).



• **Figure 2.10** Serum bile acid levels of healthy subjects and patients with alcohol-induced fatty liver and alcoholic cirrhosis. The overlap in individual values among the three groups is evident. Serum bile acids, shown on the *ordinate*, were determined by means of combined gas–liquid chromatography and mass spectrometry with deuterium-labeled internal standards. (From Einarsson K, Angelin B, Bjorkhem I, et al. The diagnostic value of fasting individual serum bile acids in anicteric alcoholic liver disease: relation to liver morphology. *Hepatology* 1985;5:108, with permission.)

P.30

Serum bile acid tests offer the advantage of being highly specific indicators of liver dysfunction. They are less sensitive than they were originally hoped to be, however, and are nonspecific in differentiating the various types of liver disease. In this respect, these tests appear similar to the BSP and ICG clearance tests, sensitive LFTs that are rarely used. They may be useful in ruling out liver disease in patients with suspected Gilbert's syndrome and Dubin-Johnson syndrome.

Breath Tests and Other Clearance Tests

The introduction of breath tests for humans in which ^{13}C- or ^{14}C-labeled aminopyrine, phenylalanine, methacetin, phenacetin, or galactose is used represents an attempt to develop a practical test of hepatic functional reserve. The principle and method of breath tests are simple. A substance labeled with ^{13}C or ^{14}C that is given orally or parenterally is converted primarily in the liver to $^{13}CO_2$ or $^{14}CO_2$. As long as absorption is complete or reproducible, the oral route is more convenient. The labeled CO_2 exhaled in the breath is collected at various intervals in an alkaline medium that serves as a CO_2 trap. Metabolism of the labeled agent by the liver can be determined semiquantitatively through the multiplication of the specific activity of exhaled labeled CO_2 over a given time interval by a value for endogenous CO_2 output of 9 mm/kg per hour.

An ideal drug for use in a breath test of hepatic drug-metabolizing capacity would have the following characteristics:

- Metabolism of the test drug should be primarily hepatic. This important parameter is often difficult to validate in humans.
- When the drug is given orally, its absorption must be rapid and complete or at least predictable.
- The drug should have a low hepatic extraction ratio (approximately 20% to 30%) so that changes in hepatic blood flow have little influence on its clearance from blood.
- The generated labeled CO_2 should be distributed evenly throughout the body and not sequestered in an unavailable compartment.
- For ease of collection of the labeled CO_2, the drug should have a short elimination half-life.
- The drug must be safe.

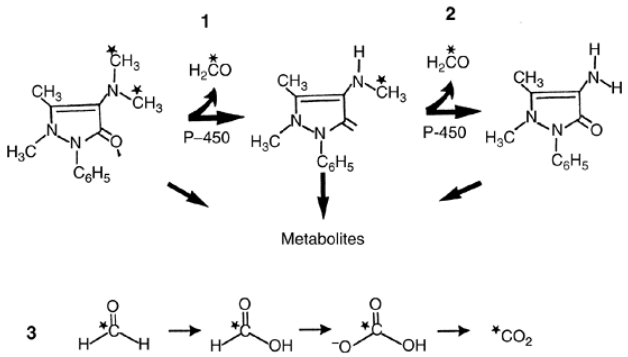
Most of these characteristics seem to have been met by the drugs in use for breath tests, although some points have not been worked out completely.

Aminopyrine breath test

There has been significant experience with the ^{13}C and ^{14}C aminopyrine breath tests. The radioactive methyl groups of aminopyrine undergo demethylation and eventual conversion through formaldehyde and formate to bicarbonate CO_2 with exhalation of labeled CO_2 in the breath (Fig. 2.11). Studies with rats have shown that the rate of appearance of $^{14}CO_2$ in the breath correlates with the mass of the hepatic mixed-function oxidase system. Studies in humans also suggest that the aminopyrine breath test is a measure of mixed-function oxidase mass because in control subjects, the rate of appearance of $^{14}CO_2$ in the breath correlates with the clearance of aminopyrine from the blood (31,32). Furthermore, the rate of appearance of $^{14}CO_2$ increases after pretreatment with phenobarbital, a known microsomal inducer, but it decreases after treatment with disulfiram, an inhibitor of the microsomal mixed-function oxidase system (31). The decreased rate of appearance of $^{14}CO_2$ in the breath also correlates well with the decreased clearance of aminopyrine from blood in patients with cirrhosis, a state in which mixed-function oxidase mass is likely to be decreased. The concept that the breath test is a measure of functioning hepatic microsomal mass is further supported by observations that low results of the aminopyrine breath test in patients with hepatocellular disease correlate well with results that reflect functional mass—the degree of prolongation of prothrombin time (33); the extent of decrease in serum albumin level (31,32); serum bile acid level (26) (Fig. 2.9); galactose elimination

P.31

capacity; and impaired BSP removal from blood. Low results of the aminopyrine breath test also correlate well with the degree of necrosis and inflammation in liver biopsy specimens from patients with alcohol-related cirrhosis (33) (Fig. 2.12). Although only 30% of the administered label is recovered in 48 hours and only 60% of the administered aminopyrine is recovered as demethylated metabolites in urine, the results of detailed analysis of the pharmacokinetics of the aminopyrine breath test support the validity of the test as a measurement of hepatic microsomal enzyme function. The clinician must be aware that factors such as diet, folate deficiency, and use of other drugs may alter the results of this test.



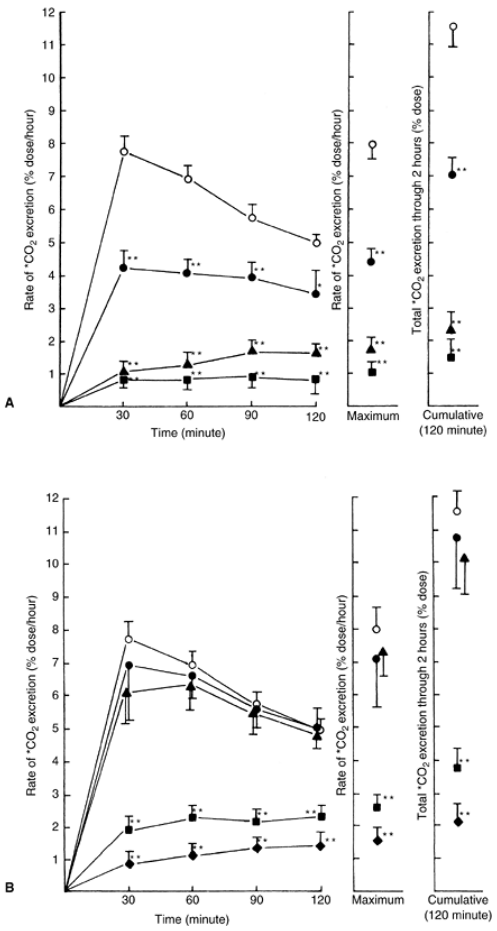
• **Figure 2.11** Metabolism of ^{14}C aminopyrine. (From Baker AI, Kotake AN, Schoeller DA, et al. Clinical utility of breath tests for the assessment of hepatic function. *Semin Liver Dis* 1983;3:318, with permission.)

The ^{14}C aminopyrine breath test is usually performed after an overnight fast. A known dose of ^{14}C aminopyrine (1 to 2 µCi) is administered orally, and breath samples are collected at 30-minute intervals for up to 4 hours. The expired CO_2 is trapped in alkali and counted. There is an excellent correlation between the percentage of administered ^{14}C expired in 2 hours (plasma clearance rate of aminopyrine) and the fractional disappearance rate of $^{14}CO_2$. Because of this, most investigators have found it satisfactory to rely on a single sample collected 2 hours after the administration of the radionuclide. Healthy persons excrete 6.6% ± 1.3% of the administered dose in the breath in 2 hours. Patients with hepatocellular injury excrete considerably less (Fig. 2.12). A single measurement at 60 minutes is more convenient for patients, yields similar data, and has been recommended (34).

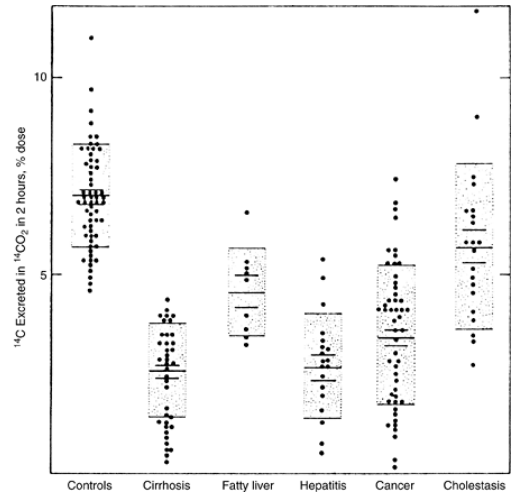
The data are clear that a single breath result cannot differentiate the types of liver disease. Levels are equally depressed in patients with alcoholic cirrhosis and severe hepatitis (34). The test may have clinical value in quantifying residual functioning liver cell mass and in establishing prognosis in diseases such as alcoholic hepatitis (34). The aminopyrine breath test appears as sensitive as the measurement of aminotransferases in the detection of hepatocellular diseases and more sensitive than the measurement of serum bilirubin, prothrombin time, and albumin. The aminopyrine breath test may be more specific in the detection of histologic severity of chronic hepatitis (35), hepatitis C (36), and alcoholic liver disease (33) than are conventional liver tests (Fig. 2.12). The breath test appears to be as sensitive as a 2-hour postprandial bile acid test, and there is a moderately good correlation between the results of the two (26). The test result is abnormal more frequently in hepatocellular than in obstructive liver disease (32), and may remain normal in advanced primary and secondary biliary cirrhosis (37). The breath test has been shown to be as good, if not better, than the Child-Pugh score or model for end-stage liver disease in predicting the likelihood of death while awaiting liver transplant (38).

The degree of depression of breath test results overlaps considerably in all types of severe liver disease, including cirrhosis, hepatitis, hepatic cancer, and various histologic forms of alcohol-related disease (32) (Fig. 2.13). In one study, the results of the aminopyrine breath test were more reliable predictors of short-term survival and clinical improvement for patients with alcoholic hepatitis than were results of conventional liver tests. There have been conflicting results in studies of the utility of the aminopyrine breath test in predicting survival among patients with cirrhosis. Results of one study suggest that the aminopyrine breath test is better than the Child-Turcotte classification for predicting survival among patients with cirrhosis who are undergoing surgery. Two other studies found the aminopyrine breath test

to be no better in prediction of survival among patients with cirrhosis than were either the serum albumin or the Child-Turcotte classification test.



• **Figure 2.12** Average excretion rate of labeled CO_2 from aminopyrine in healthy subjects and in patients with alcoholic liver disease and chronic active liver disease. **A:** *Open circles* indicate healthy subjects; *closed circles*, mild alcoholic hepatitis; *triangles*, moderate alcoholic liver disease; *squares*, severe alcoholic liver disease. *Bars* indicate standard error of the mean (SEM) statistical difference from normal controls: $P < 0.05$; $P < 0.01$. **B:** *Open circles* indicate healthy subjects; *closed circles*, chronic persistent hepatitis; *triangles*, chronic active hepatitis; *squares*, chronic active hepatitis with bridging necrosis; *diamonds*, chronic active hepatitis with cirrhosis. Other symbols are the same as in **A**. (From Schoeller DA, Baker AL, Monroe PS, et al. Comparison of different methods expressing results of the aminopyrine breath test. *Hepatology* 1982;2:455, with permission.)



• **Figure 2.13** Percentage of administered ^{14}C excreted as $^{14}CO_2$ in the breath 2 hours after oral administration of ^{14}C aminopyrine. *Transverse lines* represent standard error of the mean (SEM); *hatched areas* represent SD. (From Hepner GW, Vesell ES. Quantitative assessment of hepatic function by breath analysis after oral administration of (^{14}C) aminopyrine. *Ann Intern Med* 1975;83:632, with permission.)

Although breath tests have been available for more than 25 years in the United States, they are still used infrequently. There are several

reasons for this: They are less convenient to perform than are simple blood tests; they were previously performed only with the radioisotope ^{14}C, which has a long half-life and is not widely used by departments of nuclear medicine; and diagnostic tests with ^{14}C cannot be performed on children. Identical testing can be done with the stable isotope ^{13}C. In the past, the use of this agent required sophisticated and expensive mass spectrometers, which are not widely available. ^{13}C can now be measured by means of nondispersive infrared spectrometry, a simpler and less expensive method that provides results that correlate well with those of both mass spectrometry and $^{14}CO_2$ techniques (39).

Other labeled drugs that have been studied extensively include phenylalanine, methacetin, and galactose. Tests with phenylalanine and methacetin provide information comparable with that from aminopyrine tests and are not discussed further.

The galactose breath test was initially investigated as a substitute for the more laborious galactose elimination capacity (see subsequent text). Galactose metabolism occurs along a cytosolic pathway independent of the cytochrome P-450 system, unlike aminopyrine and methacetin. This independence results in a less metabolic variation due to drug interactions or genetic polymorphisms. Studies have shown that the results of the galactose breath test correlate well with galactose elimination capacity, are altered early in the course of hepatitis C infection, and correlate with the degree of liver fibrosis.

Because of the results of these and other studies, breath tests have been suggested as possible substitutes for liver biopsy in the evaluation of patients with chronic viral hepatitis and alcoholic hepatitis. There is little evidence, however that they will replace liver biopsy, which results in a specific diagnosis, allows assessment of the extent of liver damage, and is a measure for assessing response to therapy. Breath tests

are useful in measuring residual functional microsomal mass and may prove helpful in for establishing the prognosis and response to therapy for certain types of liver disease. However, there is little evidence that breath tests are significantly more sensitive or specific than are the currently used LFTs.

Galactose clearance

Galactose clearance has been used to measure functional hepatic mass. Galactose is given intravenously at a dose of 0.5 g/kg. Serial blood samples are collected over 60 to 90 minutes and assayed for galactose. The results are corrected for urinary galactose excretion. Patients with cirrhosis and chronic hepatitis have markedly low galactose clearance compared with healthy controls, but galactose clearance is rarely abnormal in patients with biliary obstruction. Neither the intravenous nor the oral galactose clearance test have been found better than the measurement of serum albumin in differentiating healthy individuals and patients with cirrhosis. Galactose clearance also appears to add little to standard laboratory studies in predicting the outcome for patients with fulminant hepatic failure or cirrhosis (40,41). The role of this easily performed but cumbersome test remains undefined.

Caffeine clearance

Caffeine clearance tests have been used to quantify functional hepatic capacity by means of assessment of the activity of cytochrome P-4501A2, *N*-acetyltransferase, and xanthine oxidase (42). Several variations in these tests have been described. In all studies, caffeine (200 to 366 mg) is taken orally. Early studies required that blood samples be drawn 1, 2, 3, 6, 12, 24 hours later, and plasma caffeine concentrations measured. In more recent studies, caffeine and its metabolites have been measured in 24-hour urine collections, in saliva, and in scalp hair. Results with these alternative methods are similar to those of the plasma clearance method. Overnight salivary caffeine clearance has been shown to correlate with ICG and galactose clearance and the aminopyrine breath test (43). The measurement of caffeine in scalp hair is an alternative means for collecting similar data (44). Concomitant tobacco use is a confounding factor in these tests, because it increases caffeine clearance (45). Increasing age and certain drugs may decrease clearance of caffeine and salivary pH and flow rate can produce variability with that sampling method (46). The results of caffeine clearance tests are similar to those of the ^{14}C aminopyrine test. A ^{13}C-caffeine breath test has been shown to be a valid indicator of plasma caffeine clearance, and correlates with hepatic dysfunction (47).

Lidocaine metabolite formation

Lidocaine is metabolized to its major metabolite monoethylglycinexylidide (MEGX) through sequential oxidative *N*-dealkylation within the hepatic cytochrome P-450 system (48). A dose of lidocaine (1 mg/kg) is given intravenously. Fifteen, thirty, and/or sixty minutes later, serum samples are taken to determine the MEGX concentration, which is measured most commonly by means of fluorescence polarization immunoassay. Other techniques used to measure MEGX include HPLC and gas–liquid chromatography, both of which are more specific for MEGX than is the fluorescence polarization immunoassay.

Numerous studies have been performed to assess the prognostic value of MEGX formation in patients with cirrhosis (49,50,51,52). Results of other studies suggest that a decline in MEGX concentration correlates well with histologic worsening in patients with chronic liver disease and may provide a noninvasive alternative to liver biopsy for following the course of disease (53,54,55). The MEGX test has also been shown to correlate with hepatic insufficiency and postoperative complications after liver resection in patients with cirrhosis (56). An active area of research has been the role of the MEGX test in identifying suitable donors for liver transplantation. In general, donor MEGX concentration 15 minutes after the infusion of lidocaine has been significantly higher in patients with good early graft function, than in those with poor early graft function. Unfortunately, there is significant overlap in the MEGX values between the two groups, making this test unsuitable for predicting liver function in individual cases. Additional studies will determine whether this safe and easily performed test has a role in the evaluation of hepatic synthetic function.

Tests for Detection of Injury to Hepatocytes (Serum Enzyme Tests)

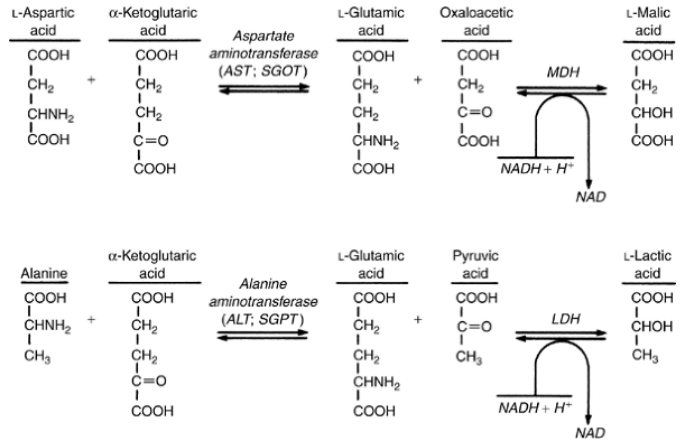
The liver contains thousands of enzymes, some of which are also present in serum in very low concentrations. These enzymes have no known function in serum and behave as other serum proteins do. They are distributed in plasma and interstitial fluid and have characteristic half-lives of disappearance, usually measured in days. Little is known about their catabolism or clearance. The elevation of activity for a given enzyme in serum is thought to primarily reflect its increased rate of entrance into serum from damaged liver cells. Serum enzyme tests can be grouped into two categories: (a) Enzymes the elevation of which in serum reflects generalized damage to hepatocytes and (b) enzymes the elevation of which in serum primarily reflects cholestasis.

Enzymes that Indicate the Presence of Hepatocellular Necrosis

Aminotransferases

The serum aminotransferases (formerly called transaminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases, such as hepatitis. The activities of alanine aminotransferase (ALT), formerly serum glutamic-pyruvic transaminase, and aspartate aminotransferase (AST), formerly serum glutamic-oxaloacetic transaminase, in serum are the most frequently measured indicators of liver disease. These enzymes catalyze the transfer of the α-amino groups of alanine and aspartic acid, respectively, to the α-keto group of ketoglutaric acid. This results in the formation of pyruvic acid and oxaloacetic acid (Fig. 2.14). Of the numerous methods developed for measuring ALT and AST activity in serum, the most specific method couples the formation of pyruvate and oxaloacetate—the products of the aminotransferase reactions—to their enzymatic reduction to lactate and malate. The reduced form of nicotinamide-adenine dinucleotide (NADH), the cofactor in this reduction, is oxidized to nicotinamide-adenine dinucleotide (NAD). Because NADH (but not NAD) absorbs light at 340 nm, the event can be followed spectrophotometrically by means of

the loss of absorptivity at 340 nm.



• **Figure 2.14** Enzymatic assay of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; MDH, malic dehydrogenase; LDH, lactate dehydrogenase; NADH, reduced nicotinamide-adenine dinucleotide; NAD, nicotinamide-adenine dinucleotide.

Both aminotransferases are normally present in serum in low concentration (<30 to 40 IU/L). The organ source of these enzymes in serum has never been firmly identified, although they probably originate in tissues rich in ALT and AST. AST is present in the liver, cardiac muscle, skeletal muscle, the kidneys, the brain, the pancreas, the lungs, leukocytes, and erythrocytes, in decreasing order of concentration ALT is present in highest concentration in the liver. The increase in the serum values of ALT and AST is related to damage to or the destruction of tissue rich in the aminotransferases or to changes in cell membrane permeability, which allows ALT and AST to leak into serum. The activity of these enzymes in serum at any moment reflects the relative rate at which they enter and leave the circulation. Injected aminotransferases are distributed in interstitial fluid as well as in plasma. From there, they are gradually cleared as other serum proteins are, AST being cleared more rapidly than ALT. The enzymes are presumably catabolized by cells in the reticuloendothelial system. Hepatic sinusoidal cells appear to be the main site for AST clearance. Almost no aminotransferases are present in urine, and only a very small amount is present in bile. It is therefore unlikely that biliary or urinary excretion plays a role in the clearance of ALT or AST.

ALT and AST both require pyridoxal 5′-phosphate as a cofactor, and may both be present in serum in apoenzyme as well as holoenzyme form (57). In tissues, ALT is present in the cytosol; whereas AST

occurs in two locations, the cytosol and mitochondria. The cytosolic and mitochondrial forms of AST are true isoenzymes and are immunologically distinct. They can be separated from each other with a number of techniques, including immunoprecipitation, chromatography, and electrophoresis. Approximately 80% of AST activity in human liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in healthy individuals is derived from the cytosolic isoenzyme. Neither ALT nor AST has isoenzymes that are tissue-specific. Hence, isoenzyme analysis of serum ALT or AST seldom is useful. Patients with acute myocardial infarction and chronic alcoholic liver disease may be exceptions. Large increases in mitochondrial AST occur in serum after extensive tissue necrosis. Because of this, assay of mitochondrial AST has been advocated as an accurate test for the detection of myocardial infarction. The level of mitochondrial AST is also increased in chronic but not acute alcoholic liver disease.

Aminotransferase levels are typically elevated in all liver disorders. These include all types of acute and chronic hepatitis, cirrhosis, infectious mononucleosis, acute and chronic heart failure, various infections, metastatic carcinoma, and granulomatous and alcoholic liver diseases (Fig. 2.15). Elevations up to eight times the upper limit of normal are nonspecific and may be found in any of the mentioned disorders. The highest elevations occur in disorders associated with extensive hepatocellular injury, such as drug and viral hepatitis, acute heart failure, and exposure to hepatotoxins such as carbon tetrachloride and phalloidin. Values are commonly in the low thousands, although values in the range of 10,000 to 15,000 IU/L (167 to 250 μkat/L[1]—see subsequent text) can occur in rare patients with viral hepatitis who make uneventful recoveries. Levels of aminotransferases are seldom elevated above 500 IU (8.34 μkat/L) in obstructive jaundice, viral hepatitis in patients with acquired immunodeficiency syndrome (AIDS), and cirrhosis (58) (Tables 2.3 and 2.4) and usually less than 300 IU (5.0 μkat/L) in alcoholic liver disease. One exception is acute bile duct obstruction caused by a common duct stone. AST and ALT values may reach the thousands within 24 to 48 hours of acute bile duct obstruction, and then rapidly decline to lower values. AST and ALT levels are equally elevated in most hepatobiliary disorders, with the ALT level usually being somewhat higher than the AST level. ALT appears to be a more sensitive and specific test of acute hepatocellular damage than AST and is commonly used in epidemiologic studies to document the incidence of viral hepatitis.

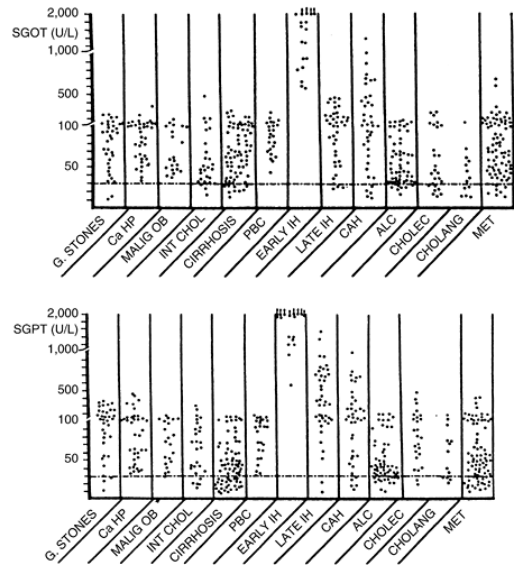
Several studies have questioned whether previously established values to define the normal ALT range are accurate, and have suggested that the upper limit of normal should be revised (59,60,61). The normal range for ALT was set in the 1950s and has changed little since then. As with most clinical laboratory tests, the normal range was defined as the mean of values from a "healthy group of individuals" ±2 SD. The healthy reference population included men and women, often medical students, blood donors, and laboratory technicians. The researchers advocating change have theorized that the "healthy reference group" possibly included patients with nonalcoholic fatty liver disease and hepatitis C, an inclusion that would have skewed the data. Researchers found that serum ALT activity was independently related to body mass index and to laboratory indicators of abnormal lipid or carbohydrate metabolism (61). They suggested lowering the upper limit of normal for men to 500 nkat/L (30 U/L) and for women to 317 nkat/L (19 U/L). Using these new values, the researchers increased sensitivity for detecting hepatitis C virus viremia from 55% to 76.3% with a drop in specificity from 97.4% to 88.5%. The benefit of this change would be marginal at best.

There is a poor correlation between the extent of liver cell necrosis and the elevation of serum aminotransferases levels. Similarly, absolute elevation of aminotransferase levels is of little value in predicting the outcome of acute hepatocellular disorders. Rapid decreases in serum aminotransferase levels are usually a sign of recovery from disease. This may be a poor prognostic sign in fulminant hepatitis, in which decreasing serum values may reflect the massive destruction and loss of viable hepatocytes.

Elevated aminotransferase activity is among the first laboratory abnormalities detected in the early phases of viral hepatitis (Fig. 2.16). In patients with jaundice due to hepatitis, the elevation of serum bilirubin level usually lags behind the increase in aminotransferase levels by approximately 1 week. Therefore, aminotransferase levels are frequently declining as bilirubin level is increasing. Aminotransferase levels typically decline steadily during recovery from viral hepatitis. Secondary increases in aminotransferase levels or

persistent elevation may indicate recrudescence of acute hepatitis or the development of chronic active hepatitis. Hepatitis C is commonly associated with fluctuations in ALT and AST levels. Many patients with persistently elevated aminotransferase levels have liver biopsy evidence of chronic hepatitis; some patients with chronic hepatitis C infection and persistently normal ALT and AST levels are found at liver biopsy to have chronic

hepatitis. The measurement of aminotransferases is one of the important means of following the clinical activity of viral hepatitis, and evaluating the response to immunosuppressive therapy for chronic hepatitis.



• **Figure 2.15** Activities of serum glutamic-oxaloacetic transaminase (SGOT; aspartate aminotransferase [AST]) and serum glutamic-pyruvic transaminase (SGPT; alanine aminotransferase [ALT]) in patients with various types of hepatobiliary disease. Upper limit of normal is shown by *dotted lines.* To convert from IU/L to µkat/L, multiply by 0.01667. G. STONES, gallstones; Ca HP, carcinoma of the head of the pancreas; MALIG OB, carcinoma of bile duct; INT CHOL, intrahepatic cholestasis; CIRRHOSIS, portal cirrhosis; PBC, primary biliary cirrhosis; EARLY IH, early infectious hepatitis; CAH, chronic active hepatitis; ALC, alcoholic hepatic disease; CHOLEC, cholecystitis; CHOLANG, cholangitis; MET, hepatic metastasis. (From Ellis G, Goldberg DM, Spooner RJ, et al. Serum enzyme tests in disease of the liver and biliary tree. *Am J Clin Pathol* 1978;70:248, with permission.)

The AST/ALT ratio is usually of little value in differentiating various hepatobiliary disorders. One important exception is the recognition of alcoholic liver disease. If the ALT is less than 300 IU (5.1 µkat/L), an AST/ALT ratio more than 2 suggests alcoholic liver disease; a ratio greater than 3 is highly suggestive of alcoholic liver disease (62) (Figs. 2.17 and 2.18). The increased ratio primarily reflects the low serum activity of ALT in patients with alcoholic liver disease (Fig. 2.19) (58). This is secondary to a deficiency of pyridoxal 5′-phosphate in patients with alcoholic liver disease (57). ALT synthesis in the liver requires pyridoxal phosphate more than does AST synthesis (57). The altered AST/ALT ratio in the serum appears to reflect the altered ratios in the liver (Fig. 2.19). The less than expected increase in serum ALT and AST values in alcoholic liver disease—usually less than 200 IU (3.3 µkat/L) and 300 IU (5.1 µkat/L), respectively—cannot be explained simply by the decreased hepatic concentrations. This becomes evident whenever a patient with alcoholic liver disease has concomitant heart failure, viral hepatitis, or drug hepatotoxicity, particularly acetaminophen. When this occurs, serum AST and ALT levels may soar to the thousands (>20 µkat/L). Despite striking elevations, the AST/ALT ratio remains increased and typical of alcoholic liver disease.

Table 2.3. Aminotransferase Values in Patients with Infectious Hepatitis and Obstructive Jaundice

Units		AST (SGOT)		ALT (SGPT)	
International (IU/L)	Infectious hepatitis SI (µkat/L)	Obstructive jaundice (Cumulative %)	Infectious hepatitis	Obstructive jaundice (Cumulative %)	
Normal		1	10	1	20
34:46[a]–200	0.57:0.77–3.33	27	81	27	60
201–400	3.35–6.67	43	98	39	93
401–600	6.68–10.00	50	99	43	97
601–800	10.01–13.34	64	99	48	99
801–1,000	13.35–16.67	72	100	53	100
1,001–2,000	16.68–33.34	95	—	83	—

2,001–3,000	33.35–50.01	99	—	91	—
3,000	50.01	100	—	100	—
Total patients		274	181	177	97

These aminotransferase values were reported in 28 papers with data on patients with infectious hepatitis and in 14 papers with data on patients with obstructive jaundice.
[a]Upper limit of normal, 33 IU/L for AST and 45 IU/L for ALT.
AST, aspartate aminotransferase (formerly serum glutamic-oxaloacetic transaminase); ALT, alanine aminotransferase (formerly serum glutamic-pyruvic transaminase); SI, Système Internationale unit.
Clermont RJ, Chalmers TC. The transaminase tests in liver disease. *Medicine* 1967;46:197, with permission.

The AST/ALT ratio has been studied as a noninvasive indicator of cirrhosis in patients with chronic hepatitis C infection (63,64,65,66). These studies have shown that an AST/ALT ratio greater than 1 indicates the presence of cirrhosis with a very high specificity (94% to 100%) but with a relatively low sensitivity (44% to 75%). The significant relation between the AST/ALT ratio and ICG clearance suggests that the increase in serum AST level that produces the increased ratio is caused by the impairment of functional hepatic blood flow in patients with cirrhosis; the result is a decrease in hepatic sinusoidal uptake of AST (65).

Researchers identified another possible contributing factor to the AST/ALT ratio seen in both alcoholics and cirrhotics (67). AST-Ig complexes were studied in 128 patients with liver disease. AST was found to bind to Ig of the IgA class, but not to either IgG or IgM. This complex was seen in 41.8% of patients with chronic hepatitis, 62.2% of patients with cirrhosis, and 66.7% of patients with alcohol-related liver disease. The high level seen in the final group likely reflected the high levels of IgA seen in patients with alcohol-related liver disease. The AST/ALT ratio was significantly higher in the patients with the AST-Ig complex, rather than those without, and this observation probably contributes to the higher AST/ALT ratio seen in patients with cirrhosis and alcoholic liver disease.

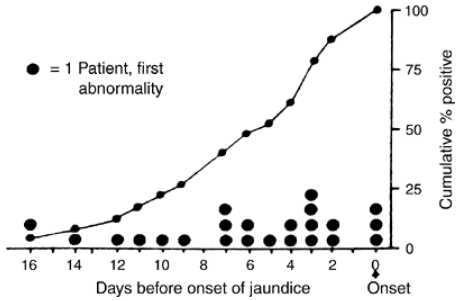
Table 2.4. Ratio of Aspartate Transferase to Alanine Transferase in Patients with High and Low Values

	Low (<500 IU/L; <8.34 µkat/L)		High (>500 IU/L; >8.34 µkat/L)		
Disorder	**<1.0[a]**	**>1.0[a]**	**<1.0[a]**	**>1.0[a]**	**Total patients**
Infectious hepatitis					
Patients	66	37	61	7	171
Percentage	64	36	90	10	—
Infectious mononucleosis					
Patients	43	7	4	0	54
Percentage	86	14	—	—	—
Obstructive jaundice					
Patients	33	22	6	1	62
Percentage	60	40	86	14	—
Cirrhosis					
Patients	4	64	1	1	70
Percentage	6	94	—	—	—
Total patients	146	130	72	9	357

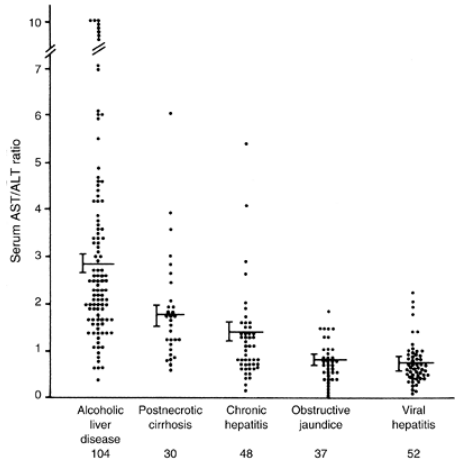
[a]Highest value of either test.
Clermont RJ, Chalmers TC. The transaminase tests in liver disease. *Medicine* 1967;46:197, with permission.

Elevated serum aminotransferase values are not specific for hepatobiliary disorders. They are also found in patients with severe cardiac and skeletal muscle damage. AST level is more often increased

in patients with myocardial infarction than ALT, and is undoubtedly of cardiac origin. Large increases in ALT in cardiac disease are probably of hepatic origin because they frequently occur among patients with large, hemodynamically significant infarcts associated with congestive heart failure and circulatory collapse. Hepatic ischemia and central hepatocellular necrosis are likely to occur in these patients.



▪ **Figure 2.16** Elevation of AST (SGOT) in the early stages of acute viral hepatitis. Relation of the first abnormality is compared with the onset of jaundice in 23 patients or volunteers closely observed during the incubation period of viral hepatitis. (From Clermont RJ, Chalmers TC. The transaminase tests in liver disease. *Medicine* 1967;46:197, with permission.)



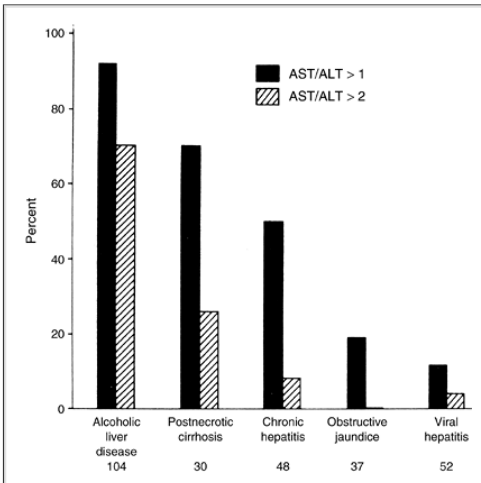
▪ **Figure 2.17** Aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio in patients with biopsy-proven liver disease. The ratio was calculated from the serum sample that had the highest individual aminotransferase value, be it AST or ALT. (Adapted from Cohen JA, Kaplan MM. The SGOT/SGPT ratio: an indicator of alcoholic liver disease. *Dig Dis Sci* 1979;24:835, with permission.)

Increased AST and ALT values in muscle disease are probably derived from muscle. The extent of enzyme elevation in muscle disease is typically less than 300 U/L (5.1 μkat/L) but has been reported much higher (68).

P.40

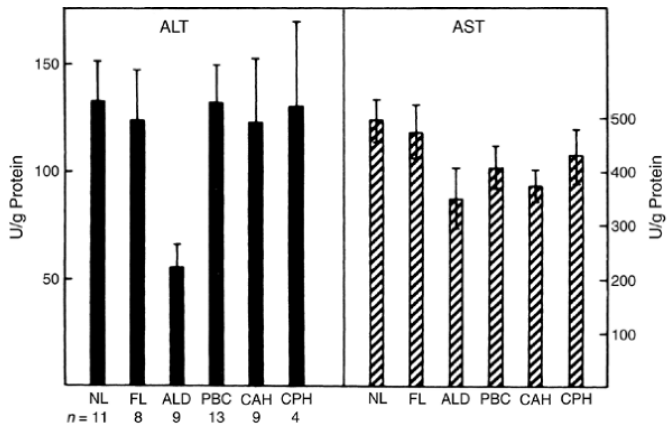
P.41

Except in instances of acute rhabdomyolysis, values rarely reach the range observed in patients with acute hepatocellular disorders. However, serum AST and, occasionally, ALT activity, can increase slightly after vigorous exercise with an AST levels of greater then 1,000 reported. In such cases the AST/ALT is initially greater than 3:1, but the ratio rapidly approaches 1:1 given the shorter half-life of AST (68). The ratio is typically close to 1:1 in patients with chronic muscle diseases or injury. This may account for the slight, usually unexplained increases in aminotransferase values observed among some runners.



▪ **Figure 2.18** Percentage of patients with aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratios greater than 1 and greater than 2. (Adapted from Cohen JA, Kaplan MM. The SGOT/SGPT ratio: an indicator of alcoholic liver disease. *Dig Dis Sci*

1979;24:835, with permission.)



• **Figure 2.19** Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in human liver. The activity of ALT is selectively decreased in livers of patients with alcoholic hepatitis, cirrhosis, or both. NL, normal liver; FL, fatty liver in the absence of alcoholism; ALD, alcoholic hepatitis, cirrhosis, or both; PBC, primary biliary cirrhosis; CAH, chronic active hepatitis; CPH, chronic persistent hepatitis. (Adapted from Matloff DS, Selinger MJ, Kaplan MM, et al. Hepatic transaminase activity in alcoholic liver disease. *Gastroenterology* 1980;78:1389, with permission.)

Levels of the aminotransferases can be falsely elevated or diminished under certain circumstances. Drugs such as erythromycin and para-aminosalicylic acid can yield falsely elevated aminotransferase values if older colorimetric tests are used. Conversely, low AST values can occur in patients with uremia. These low values increase after dialysis; this finding suggests that a dialyzable inhibitor of the aminotransferase reaction is present in the serum of patients with uremia.

Other Enzyme Tests of Hepatocellular Necrosis

A number of other serum enzyme tests have been promulgated as being either more specific or more sensitive in the detection of hepatocellular necrosis than is the measurement of aminotransferases. Some of these enzymes are only found in liver tissues and, theoretically, the tests should be more specific for liver disease than are the aminotransferase tests. None of them has proved more useful in practice than the aminotransferase tests, and none are more widely used. A brief description of some of these enzyme tests follows.

Glutamate dehydrogenase

Glutamate dehydrogenase, a mitochondrial enzyme, is present primarily in the liver, heart, muscle, and kidneys. In the liver, it is present in its highest concentration in the centrilobular hepatocytes. Because of this location and the fact that the level is particularly elevated after acute right-sided heart failure, serum glutamate dehydrogenase was investigated as a specific marker for liver disorders that primarily affect centrilobular hepatocytes, such as alcoholic hepatitis. An initial report that glutamate dehydrogenase may be a sensitive and a relatively specific marker for alcoholic hepatitis has not been confirmed by others. Glutamate dehydrogenase is seldom used as an LFT.

Isocitrate dehydrogenase

Isocitrate dehydrogenase (ICDH), a cytoplasmic enzyme, is present in the liver, heart, kidneys, and skeletal muscle. Its activity in serum parallels that of the aminotransferases in acute and chronic hepatitis, but it is less sensitive. Although elevations in serum ICDH are relatively specific for liver disorders, increased levels have also been reported in disseminated malignant disease without detectable hepatic involvement. Like glutamate dehydrogenase, ICDH is predominantly found in hepatocytes in the centrilobular zone. It has been advocated as a possible marker of centrilobular necrosis (69). The measurement of ICDH does not yet offer any diagnostic advantage over the measurement of aminotransferases.

Lactate dehydrogenase

Lactate dehydrogenase is a cytoplasmic enzyme present in tissues throughout the body. Five isoenzymes of lactate dehydrogenase are present in serum, and are readily separated by various electrophoretic techniques. The slowest migrating band predominates in the liver. This test is not as sensitive as aminotransferase tests in the detection of liver disease and has poor diagnostic specificity, even when isoenzyme analysis is used. The lactate dehydrogenase test is more useful as a marker of myocardial infarction and hemolysis.

Sorbitol dehydrogenase

Sorbitol dehydrogenase is a cytoplasmic enzyme predominantly present in the liver; only relatively low concentrations are found in the prostate gland and kidneys. The activity of sorbitol dehydrogenase in serum parallels that of the aminotransferases in hepatobiliary disorders. The measurement of sorbitol dehydrogenase appears to be less sensitive, however, and values may be normal in cirrhosis and other chronic liver disorders. The instability of this enzyme in serum further limits its diagnostic usefulness.

Enzymes for the Detection of Cholestasis

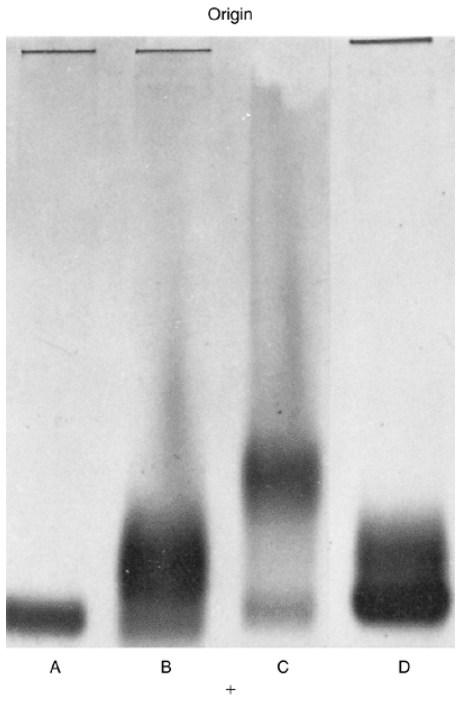
Alkaline phosphatase

Alkaline phosphatase is the name given to a group of enzymes that catalyze the hydrolysis of a large number of organic phosphate esters, optimally at an alkaline pH. Inorganic phosphate and the organic radical are generated by the reaction. The alkaline phosphatases of different tissues are true isoenzymes because they catalyze the same reaction, but differ in certain physicochemical properties (70). At least three separate genes code for the alkaline phosphatases. Most of the alkaline phosphatase isoenzymes of clinical interest are from the liver, bone, first-trimester placenta, and kidneys, and are coded by one gene. They are called *tissue-unspecific alkaline phosphatase.* They have

the same immunologic properties and amino acid sequence. Their unique physicochemical properties are conferred by the different carbohydrate and lipid side chains added by posttranslational modification. A second gene codes for third-trimester placental and intestinal alkaline phosphatase, and a third gene codes for a second intestinal alkaline phosphatase.

The precise function of alkaline phosphatase is unknown. Alkaline phosphatases are found in many locations throughout the body, including bone osteoblasts, the canalicular membranes of hepatocytes, the brush border of the mucosal cells of the small intestine, the proximal convoluted tubules of the kidney, the placenta, and white blood cells (53). In rat liver, alkaline phosphatase appears to have an active role in down-regulating the secretory activities of the intrahepatic biliary epithelium (71). In bones, the enzyme appears to be concerned with calcification, although its precise function is unknown. At other sites, it may participate in transport processes, but its actual physiologic purpose is largely unknown. Alkaline phosphatase activity is normally demonstrable in serum. There is good evidence that serum enzyme in the normal adult is primarily derived from three sources—the liver, bone, and in some instances, the intestinal tract (Fig. 2.20). The liver and bone are the major sources (70). Contribution from the intestine (approximately 10% to 20%) is of importance, primarily in individuals with blood groups O and B who secrete the ABH red blood cell antigen, and the blood level of alkaline phosphatase is enhanced after the consumption of a fatty meal (72). Studies with infused placental alkaline phosphatase have shown that the circulating enzyme appears to behave as other serum proteins do. The half-life is 7 days, and clearance from the serum is independent of the functional capacity of the liver or the patency of the bile ducts. The sites of degradation are unknown.

Some details of the more popular procedures used to measure alkaline phosphatase activity, including substrates used, end products measured, and the usual range of normal values for adults 17 to 55 years of age, are contained in Table 2.5 (70). In the most widely used procedure ρ-nitrophenylphosphate is used as a substrate and an amino alcohol, such as 2-amino-2-methyl-1-propanol, is used as a buffer. The rate of release of ρ-nitrophenol or phosphate from the substrate is measured under specified incubation conditions. The results are expressed in international units (IU/L), which is the activity of alkaline phosphatase that releases 1 mmol of chromogen or inorganic phosphate (P_i) per minute. Results obtained with these methods appear to be equally effective in the detection of a variety of clinical diseases. Use of conversion factors allows the interchange of values obtained with the different methods. These factors are based on average values, however, and correlate poorly in individual patients. This is not surprising because serum alkaline phosphatase consists of isoenzymes that differ in reactivity in various assay systems (70). A simple way of comparing the results of different methods is to express the results as multiples of the upper limit of normal. A number of analytic sources of error exist. Factors such as the concentrations of phosphate, citrate, and magnesium, and the type and concentration of the buffer may be important.



• **Figure 2.20** Alkaline phosphatase isoenzymes separated in 7.5% polyacrylamide gel slabs at pH 7.5. *A*, liver isoenzyme from a patient with obstructive jaundice; *B*, bone isoenzyme from a patient with Paget's disease of bone; *C*, liver and intestinal isoenzymes from a patient with alcoholic cirrhosis; *D*, liver and bone isoenzymes from a patient with celiac sprue and osteomalacia. (From Kaplan MM. Alkaline phosphatase. *Gastroenterology* 1972;62:452, with permission.)

In the 15- to 50-year-old age-group, mean serum alkaline phosphatase activity is somewhat higher in men than in women (Fig. 2.21). In contrast, among individuals older than 60 years, the enzyme activity of women equals or exceeds that of men, and both sexes have somewhat higher values than younger adults (73). The reasons for these differences are not known. In children, serum alkaline phosphatase activity is

considerably elevated in both sexes, correlates well with the rate of bone growth, and appears to be accounted for by the influx of enzyme from osteoid tissue. Serum alkaline phosphatase in healthy adolescent males may reach mean levels three times greater than in healthy adults, without implying the presence of hepatobiliary disease. Enzyme activity in serum may double late in normal pregnancy, primarily because of the influx of placental phosphatase.

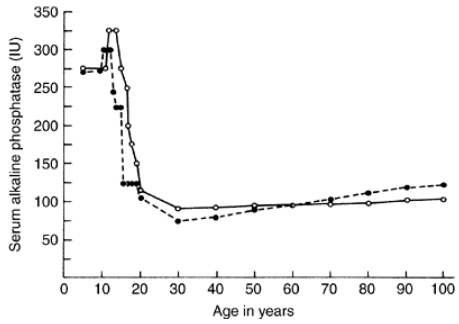
Table 2.5. Clinical Methods of Serum Alkaline Phosphatase Determination

Method	Substrate (μmol/mL)	Temperature (°C)	Buffer pH	Unit	Normal range

Bessey-Lowry-Brock	*p*-Nitrophenylphosphate (5.4)	39	10.5 Glycine	1 µmol/L *p*-nitrophenol per 60 min	0.8–3.0
Bodansky	β-Glycerophosphate (14.5)	37	8.6 Diethyl barbiturate	1 mg/100 mL inorganic phosphate per 60 min	1.5–4.0
International	*p*-Nitrophenylphosphate (2.8)	37	10.5 2-Amino-2-methyl-1-propanol	1 µmol/L *p*-nitrophenol per min	21.0–85.0
International	Phenylphosphate (9.2)	37	10.0 Sodium carbonate	1 mg/100 mL phenol per 30 min	3.0–13.0
King-Armstrong	Phenylphosphate (4.75)	37	9.3 Diethyl barbiturate	1 mg/100 mL phenol per 30 min	3.0–13.0
Klein-Read-Babson	Phenolphthalein diphosphate (2.5)	37	9.3 Tris	1 mg/100 mL phenolphthalein per 30 min	1.0–4.0
Shinowara-Jones-Reinhart	β-Glycerophosphate (3.2)	37	9.3 Diethyl barbiturate	1 mg/100 mL phenol per 60 min	2.2–8.6

Tris, tris (hydroxymethyl) aminomethane.
Adapted from Kaplan M. Alkaline phosphatase. *Gastroenterology* 1972;62:452, with permission.

Although the elevation of serum alkaline phosphatase activity is common in various hepatobiliary diseases, similar elevations are observed in disorders of bone characterized by increased osteoblastic activity, and normally occur during growth and pregnancy. The intestinal tract may occasionally and the kidneys may rarely be the sources of an elevated serum enzyme level (70,74).



• **Figure 2.21** Normal serum alkaline phosphatase values at various ages for men (*open circles*) and women (*closed circles*). (Multiply by 0.01667 to convert to SI units, µkat/L.) (Data derived from Clarke LC, Beck EJ. Plasma "alkaline" phosphatase activity, I: normative data for growing children. *J Pediatr* 1950;36:335, and Wolf PL. Clinical significance of an increased or decreased serum alkaline phosphatase level. *Arch Pathol Lab Med* 1978;102:497.)

If an elevated serum alkaline phosphatase level is the only abnormal finding in an apparently healthy individual, or if the degree of elevation is higher than expected in the clinical setting, the identification of the source of elevated isoenzyme is helpful.

This problem can be approached in several ways. First, and most precise, is the fractionation of the alkaline phosphatase by electrophoresis (75). Alkaline phosphatases derived from the liver, bone, intestines, and placenta have differing electrophoretic mobilities. In most clinical situations, this amounts to separating the liver from bone alkaline phosphatase (76). In a study in a university hospital in which the subjects were 317 patients selected because they had elevated serum alkaline phosphatase activity, the liver isoenzyme was the source of the elevation in 253 patients, bone in 58 patients, a mixture of bone and liver in 4 patients, and the intestine in only 2 patients (76). Unfortunately, the bone and liver isoenzymes only differ slightly in electrophoretic mobility. These isoenzymes often overlap if the test is run on the electrophoretic systems used in most routine clinical laboratories. Separation on polyacrylamide gel slabs is the most reliable method and produces clear-cut separations of the liver, bone, intestinal, and placental isoenzymes (Fig. 2.20). This method is not always available, however. Electrophoresis on cellulose acetate with the addition of heat inactivation may accomplish the same purpose.

The second approach is based on the observation that alkaline phosphatases from individual tissues differ in susceptibility to inactivation by heat or 2 mol urea (70). Placental alkaline phosphatase and an isoenzyme present in certain cancers—the Regan isoenzyme—are fully heat-stable after exposure to a temperature of 56°C for 15 minutes. The enzymes derived from bone, the intestines, and the liver are partly inactivated. Accordingly, the finding of an elevated serum alkaline phosphatase level in a patient with all excess activity in a heat-stable fraction, strongly suggests that the placenta or a tumor is the source of the elevation of the enzyme level in serum. Susceptibility to inactivation by heat and urea increases, in order, for intestinal, liver, and bone alkaline phosphatases, bone being by far the most sensitive (70).

Unfortunately, in evaluations of unselected patients, both heat inactivation and 2 mol urea were not found diagnostically useful. A confounding factor in the use of heat inactivation is that slight changes in temperature—as little as 0.2°C—substantially alter the rates of inactivation (70). The presence of more than one alkaline phosphatase isoenzyme in serum, each with its own rate of heat denaturation, may also yield results that cannot be interpreted. We have not found these methods useful and do not recommend them.

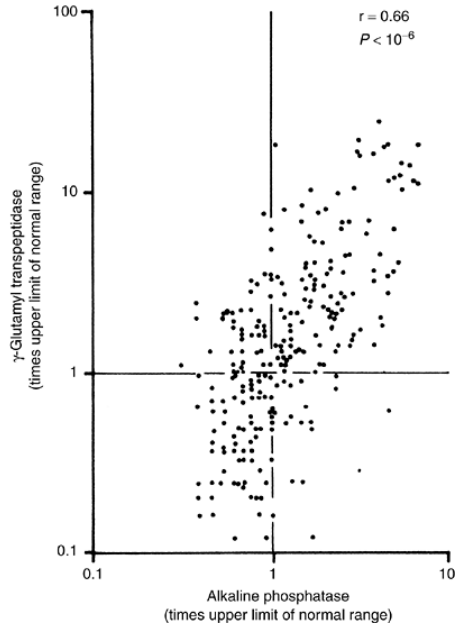
In the third and best substantiated approach, the activity of serum, leucine aminopeptidase, 5′-nucleotidase, and γ-glutamyl transpeptidase (GGT) is measured (Fig. 2.22). The levels of these enzymes, discussed later, are not elevated in bone disorders but only with liver dysfunction or, in the case of leucine aminopeptidase and possibly 5′-nucleotidase, in pregnancy. An increase of these enzymes in the serum of nonpregnant patients indicates that an elevated serum alkaline phosphatase level is caused at least in part by hepatobiliary disease. In contrast, the lack of an increased level of 5′-nucleotidase in serum in the presence of an elevated level of alkaline phosphatase does not rule out liver disease, because these enzymes do not necessarily increase in a parallel manner in early or modest hepatic injury.

In all patients who have been evaluated, elevated levels of alkaline phosphatase in serum have originated in tissues with either functionally disturbed metabolism (obstructed liver) or greatly stimulated metabolism (third-trimester placenta and bone in growing children). There is general agreement regarding the skeletal origin of the elevated serum alkaline phosphatase level in patients with bone disorders, and in growing children and the placental origin in women during the third trimester of pregnancy. Only with reference to patients with hepatobiliary disorders have there been any questions regarding the mechanism of the increased serum alkaline phosphatase level. The following two theories had been proposed: (a) The damaged liver regurgitates hepatic alkaline phosphatase back into the serum, and (b) the damaged liver, particularly if the damage is caused by obstructive jaundice, fails to excrete alkaline phosphatase made in bone, the intestines, and the liver.

This long-standing debate was resolved in favor of the former: The regurgitation of liver alkaline phosphatase into serum. The data supporting this theory are compelling. First, only hepatic alkaline phosphatase is found in the serum of patients with liver disease, particularly cholestasis (75,76). Second, the clearance rates of infused placental alkaline phosphatase are the same in patients with bile duct obstruction and in healthy individuals. Third, in experimental models of bile duct obstruction in rats, the entire increase in serum alkaline phosphatase activity is caused by the leakage of hepatic alkaline phosphatase into serum (77). The increased serum activity is paralleled by a striking increase in hepatic alkaline phosphatase activity. The increased hepatic activity cannot be accounted for by the biliary retention of alkaline phosphatase (77).

The mechanism by which hepatobiliary disease leads to an elevation of serum alkaline phosphatase has been greatly clarified. Most evidence suggests that this elevation primarily occurs because of de novo synthesis of the enzyme in the liver, and release of the phosphatase into the circulation (77,78). This process appears to be mediated by the action of bile acids,

which induce the synthesis of the enzyme and may cause it to leak into the circulation, perhaps by means of disruption of hepatic organelles and solubilization of phosphatase bound to such membranes (78). The precise manner in which the phosphatase reaches the circulation is unclear. In some patients with cholestasis, small vesicles that contain many basolateral (sinusoidal) membrane enzymes—including alkaline phosphatase—still bound to these membranes have been found in serum (79).



• **Figure 2.22** Correlation between serum γ-glutamyl transpeptidase and serum alkaline phosphatase levels in 245 healthy subjects and patients with hepatobiliary diseases. The units on the abscissa and ordinate are the logarithms of the multiples of the upper limits of normal for each test. Each point represents one patient. Although the correlation between the logarithmic values of the population (r = 0.66) is good, a considerable variation exists between the percentage of γ-glutamyl transpeptidase and alkaline phosphatase elevations in individual patients. (From Whitefield JB, Pounder RE, Neale G, et al. Serum γ-glutamyl transpeptidase activity in liver disease. *Gut* 1972;13:702, with permission.)

The principal value of serum alkaline phosphatase in the diagnosis of liver disorders is in recognition of cholestatic disorders. Approximately 75% of patients with prolonged cholestasis have alkaline phosphatase values increased fourfold or greater. Such elevations occur in both extrahepatic and intrahepatic obstruction, and the extent of the elevation does not differentiate the two. There is essentially no difference among the values found in obstructive jaundice due to cancer, common duct stone, sclerosing cholangitis, or bile duct stricture. Values are similarly increased in patients with intrahepatic cholestasis due to drug-induced hepatitis, primary biliary cirrhosis, rejection of transplanted liver, and, rarely, alcohol-induced steatonecrosis. Values are also greatly elevated in hepatobiliary disorders in patients with AIDS (e.g., primary sclerosing cholangitis due to cytomegalovirus infection and tuberculosis with hepatic involvement) (7).

Lesser increases in alkaline phosphates activity—up to three times the upper limit of normal—are nonspecific and may occur in all types of liver disorders—including viral hepatitis, chronic hepatitis, cirrhosis, infiltrative diseases of the liver—and congestive heart failure.

Isolated elevations of hepatic alkaline phosphatase or disproportionate elevation compared with results of other tests, such as the measurement of aminotransferases and serum bilirubin, may occur in partial bile

duct obstruction due to gallstones or tumor, as well as in infiltrative disease, such as sarcoidosis, hepatic abscesses, tuberculosis, and metastatic carcinoma (70). The mechanism is unknown, but probably represents local areas of bile duct obstruction with induction and leakage into serum of hepatic alkaline phosphatase from these obstructed areas. An elevated serum alkaline phosphatase level in patients with primary extrahepatic cancer does not necessarily imply metastasis to the liver or bone. Some cancers secrete their own alkaline phosphatase into serum or cause leakage of hepatic alkaline phosphatase into serum by an unknown mechanism (76).

Moderate elevations in the level of alkaline phosphatase of hepatic origin can occur in disorders that do not directly involve the liver, such as stage I or II Hodgkin's disease, myeloid metaplasia, congestive heart failure, intra-abdominal infections, and osteomyelitis (76). Certain families may also have increased levels of serum alkaline phosphatase that are genetic in origin (74). Finally, extremely low levels of alkaline phosphatase can be present in patients with fulminant Wilson disease complicated by hemolysis (80,81).

5′-Nucleotidase

5′-Nucleotidase specifically catalyzes the hydrolysis of nucleotides such as adenosine 5′-phosphate and inosine 5′-phosphate, in which the phosphate is attached to the 5 position of the pentose moiety. 5′-Nucleotidase is found in the liver, intestines, brain, heart, blood vessels, and endocrine pancreas. In the liver, the enzyme is primarily associated with canalicular and sinusoidal plasma membranes. The physiologic purpose is unknown. In most laboratories, 5′-nucleotidase activity is assayed by the use of adenosine 5′-phosphate as substrate, and measurement of either the released inorganic phosphate or the free adenosine. The presence of alkaline phosphatase in serum complicates the assay because it also hydrolyzes the 5′-nucleotide substrates. Corrections for alkaline phosphatase activity can be made in two ways: (a) Preliminary incubation of serum with appropriate concentrations of ethylenediaminetetraacetic acid, which selectively inactivates only alkaline phosphatase or (b) assay in the presence and absence of nickel (Ni^{2+}), a heavy metal that specifically inhibits 5′-nucleotidase. In most clinical laboratories, free inorganic phosphate is measured. A unit of 5′-nucleotidase activity is designated as an equivalent to the amount of enzyme that liberates 1 mg of phosphate per 100 mL of serum per hour. These units are analogous to the old Bodansky units of alkaline phosphatase activity and are expressed as such. In most series of healthy adults, the serum 5′-nucleotidase level ranges from 0.3 to 3.2 Bodansky units, and is not clearly influenced by sex or race. Values are substantially lower in children than in adults, increase gradually with adolescence, and reach a plateau after 50 years of age.

Serum values of 5′-nucleotidase are primarily elevated in hepatobiliary disease with a spectrum of abnormal values similar to that for alkaline phosphatase. The parallel behavior of these two enzymes in hepatobiliary disease probably reflects their similar subcellular location in hepatocytes (82). Both enzymes are bound to bile canalicular and sinusoidal membranes, and must be solubilized to gain access to the circulation. Bile acids may act as detergents and solubilize them. In experimental bile duct obstruction in rats, bile acid concentrations rapidly reach levels sufficient to disrupt plasma membranes and solubilize both these enzymes. The same may occur in hepatobiliary disorders in which any degree of cholestasis develops (82).

Results of most studies indicate alkaline phosphatase and 5′-nucleotidase are equally valuable in demonstrating biliary obstruction or hepatic infiltrative and space-occupying lesions. In selected patients, however, the level of one enzyme may be elevated and that of the other normal. Although the coefficient of correlation between the two enzymes is high, the values may not increase proportionately in individual patients (83).

Most data suggest that the 5′-nucleotidase and serum alkaline phosphatase are of equal value in differentiating obstructive from parenchymal liver disease. All investigators have shown some overlap in 5′-nucleotidase values in obstructive and hepatocellular jaundice. Some investigators have found this overlap to be small, and have concluded that this assay is equal to or better than the measurement of serum alkaline phosphatase for differentiating these two types of jaundice. Others have reported that the measurement of alkaline phosphatase has greater selective value (83).

Conflicting data have been reported for values of 5′-nucleotidase activity in serum during normal pregnancy. Some investigators have found an increase in enzyme activity in the third trimester, and others report no change during pregnancy. It is not clear whether these experiences are accounted for by differences in methods used to measure 5′-nucleotidase activity. The major advantage of 5′-nucleotidase over the nonspecific alkaline phosphatase measurement in serum is enhanced specificity. Most studies show that the serum level of 5′-nucleotidase does not increase in bone disease; in the few instances in which an increase was observed, it was of low magnitude (83). This is in striking contrast to results with alkaline phosphatase (83).

The greatest value of the 5′-nucleotidase assay is its specificity for hepatobiliary disease. An increased serum 5′-nucleotidase level in a nonpregnant individual

suggests that a concomitantly increased serum alkaline phosphatase level is of hepatic origin. A normal nucleotidase level in the presence of an elevated serum alkaline phosphatase level does not rule out the liver as the source of the elevated phosphatase level. The level of one enzyme may occasionally be normal and the other elevated in liver disease.

γ-Glutamyl transpeptidase

GGT catalyzes transfer of the γ-glutamyl group from γ-glutamyl peptides such as glutathione to other peptides and to L-amino acids. γ-L-Glutamyl-ρ-nitroanilide is most commonly used as a substrate in its assay with glycylglycine as the acceptor. The enzyme catalyzes the transfer of the γ-glutamyl moiety from the substrate to glycylglycine and therefore liberates the chromogen ρ-nitroaniline, which can be measured spectrophotometrically. GGT is present in cell membranes in many tissues, including the kidneys, pancreas, liver, spleen, heart, brain, and seminal vesicles. It is thought to play a role in amino acid transport across membranes as part of the γ-glutamyl cycle. Some data suggest that hydrolysis of glutathione rather than transpeptidation may be its true physiologic function. The enzyme is present in normal human serum. Serum enzyme values are usually comparable for men and women, although some investigators have found higher values in men (84). Children more than 4 years have serum values of healthy adults. Serum enzyme activity does not increase during the course of normal pregnancy. The normal range is 0 to 30 IU/L (0 to 0.5 μkat/L).

Elevated serum enzyme activity is found predominantly and in high frequency in diseases of the liver, biliary tract, and pancreas (84). Abnormal values appear in approximately the same spectrum of hepatobiliary diseases as for alkaline phosphatase, leucine aminopeptidase, and 5′-nucleotidase. Some investigators find the GGT test more sensitive than alkaline phosphatase and leucine aminopeptidase tests in the detection of liver disease (84). Others find little difference in sensitivity between GGT and alkaline phosphatase tests. A reasonably good, albeit far from perfect, correlation exists between GGT levels and those of 5′-nucleotidase and alkaline phosphatase in liver disease (Figs. 2.22 and 2.23).

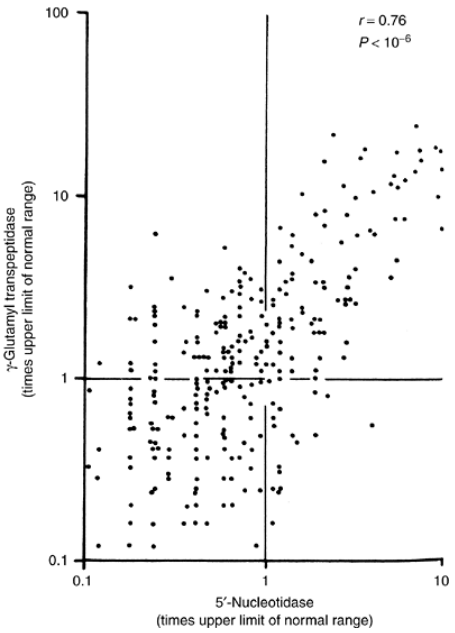
The clinical value of GGT lies in its use in conferring organ specificity to an elevated value of alkaline phosphatase, because GGT activity is not elevated in patients with bone disease. In addition, high GGT values are found in patients who take medications such as barbiturates or phenytoin (Dilantin), or ingest large quantities of alcohol (85,86), even when other serum enzyme and bilirubin values are normal. When the elevated GGT value is associated with the use of anticonvulsant drugs or alcohol abuse, no correlation between serum GGT and alkaline phosphatase values is seen. Some investigators have found that an isolated elevation in GGT level, or an elevation in GGT level out of proportion with that of other enzymes such as alkaline phosphatase or ALT is an indicator of alcohol abuse or alcoholic liver disease (Fig. 2.24). The induction of hepatic microsomal GGT by alcohol and other drugs may account for some of these observations. This is not the only explanation, however, because neither elevated serum GGT levels, nor a history of recent alcohol ingestion correlates with hepatic GGT activity in patients with biopsy-proven alcoholic liver disease (87). In addition, the alkaline phosphatase and hepatic GGT activities are similarly increased in patients with alcoholic hepatitis, yet serum GGT levels were 1,300% of normal while alkaline phosphatase serum levels were only slightly above normal (Fig. 2.24) (87). Results of in vitro studies suggest that alcohol may also cause the leakage of GGT from hepatocytes.

Normal GGT values in childhood and in pregnancy offer additional diagnostic possibilities for this test, although little has been published about clinical experience with GGT determinations in liver disease during these states. Elevation of GGT activity in serum may be depressed by female sex hormones. This was inferred from reports that GGT activity was increased less frequently and to a lesser degree in women who contracted viral hepatitis during the latter half of pregnancy or while taking oral contraceptives. In the latter study, it was also found that hyperbilirubinemia interfered with the measured activity of GGT in vitro. Both factors seem to operate in other liver disorders and undoubtedly contribute to the impaired differential diagnostic usefulness of the GGT enzyme test. Aside from its value in conferring liver specificity to an elevated alkaline phosphatase level and its possible use in identifying that a patient abuses alcohol, GGT testing offers no advantage over aminotransferase and alkaline phosphatase testing. In a prospective study with 1,040 nonselected inpatients, 139 patients (13.4%) had elevated serum GGT activity. Only 32% of these patients had hepatobiliary disease; the other 68% had other diseases that did not involve the liver (88).

Leucine aminopeptidase

Leucine aminopeptidase, a proteolytic enzyme, hydrolyzes tissue amino acids from the *N*-terminal of proteins and polypeptides. It is most active when leucine is the *N*-terminal residue; hence, its name. Leucine aminopeptidase is found in all human

tissues assayed; it has high activity in the liver, where it is primarily localized in the biliary epithelium. The function of this enzyme is not known, although it possibly involves hydrolysis of a peptide bond near an L-leucine residue, or the transfer of L-leucine from one peptide to another. The leucine aminopeptidase of normal serum, as a rule, is electrophoretically homogenous and probably originates in the liver. In hepatobiliary disease, several peaks of activity are detected, and they probably represent isoenzymes.

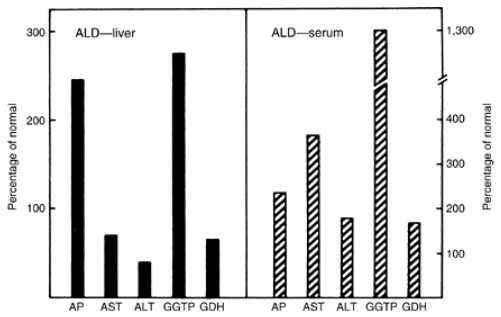


• **Figure 2.23** Correlation between serum γ-glutamyl transpeptidase and serum 5′-nucleotidase values in 245 healthy subjects and patients with hepatobiliary diseases. The units on the abscissa and ordinate are the logarithms of the multiples of the upper limits of normal for each test. Although the correlation between the logarithmic values of the population (r = 0.76) is good, there is considerable variation between the percentage of γ-glutamyl transpeptidase and 5′-nucleotidase elevations in individual patients. (From Whitefield JB, Pounder RE, Neale G, et al. Serum γ-glutamyl transpeptidase activity in liver disease. *Gut* 1972;13:702, with permission.)

The prevalent method for measuring serum leucine aminopeptidase involves α-leucyl-β-naphthylamine hydrochloride as a substrate; the liberated β-naphthylamine is assayed colorimetrically. Some evidence exists that the peptidase responsible for this reaction differs from the peptidases that hydrolyze other leucine compounds. Accordingly, in extrapolating data from one study to another, one should carefully consider the substrate used. Normal values when α-leucyl-β-naphthylamine is used usually range from 50 to 220 IU without a significant difference due to sex, age (18 to 75 years), or fed state.

Elevation in the leucine aminopeptidase level, like that of 5′-nucleotidase and to a lesser degree GGT, appears to be specific for liver disorders. The level of leucine aminopeptidase is not elevated in patients with bone disease (89), and enzyme values in children, although based on a small number of determinations, are comparable with those of adults. The only condition other than hepatobiliary disease known to result in an increase in this enzyme is pregnancy (90). The level of serum leucine aminopeptidase progressively increases during gestation and reaches a peak at term. After delivery, the enzyme level falls, decreasing approximately 35% in 4 days. Electrophoresis of serum leucine aminopeptidase from pregnant women and from individuals with liver disease shows much overlap

among the isoenzymes. This procedure is probably of no practical value in differentiating these two sources of the enzyme.



• **Figure 2.24** Enzyme values in liver tissue and serum of patients with biopsy-proven alcoholic hepatitis and cirrhosis (ALD). Values are expressed as a percentage of the values in healthy subjects. Alkaline phosphatase (AP) and γ-glutamyl transpeptidase (GGTP) activities are similarly elevated in the liver tissue of patients with ALD, but serum GGTP level is 1,300% of normal, whereas serum AP level is only 240% of normal. The data suggest that induction of hepatic GGTP by alcohol is not the sole or major cause of the serum GGTP elevation. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GDH, glutamate dehydrogenase. (From Kaplan MM. Biochemical basis of serum enzyme abnormalities in alcoholic liver disease. In: Chang NC, Chao HM, eds. Early identification of alcohol abuse. *NIAAA Res Monogr* 1985;17:186, with permission.)

Serum leucine aminopeptidase testing is as sensitive as the measurement of alkaline phosphatase and 5′-nucleotidase in the detection of obstructive, infiltrative, or space-occupying lesions of the liver. Some investigators consider leucine aminopeptidase a more sensitive indicator of infiltrative diseases, rather than alkaline phosphatase in nonjaundiced patients (89). Contrary to the findings described in the original reports, pancreatic cancer without hepatobiliary disease does not cause an elevation in the level of serum leucine aminopeptidase.

Leucine aminopeptidase activity is elevated in most types of liver disease, but values are highest in biliary obstruction. Some investigators have promulgated it as a reliable test for differentiating obstructive and hepatocellular liver disease (86). Others have observed a considerable overlap in values among patient groups, and found serum alkaline phosphatase to be at least as selective. The controversy regarding the specificity of measurement of leucine aminopeptidase as a test of biliary obstruction has never been resolved. Because of the availability of other equally sensitive, convenient, and specific tests, leucine aminopeptidase is not widely used. Its possible value is its specificity only for liver disease. In this regard, 5′-nucleotidase and GGT seem to have comparable merit.

Tests of the Biosynthetic Capacity of the Liver

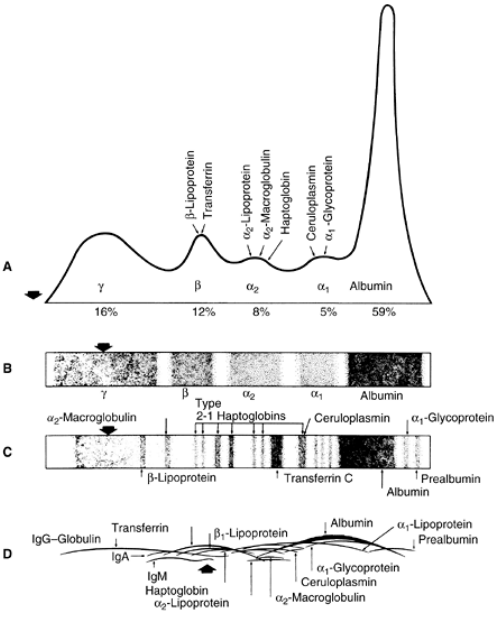
Serum Proteins

Serum contains a complex mixture of proteins that have been extensively studied with various techniques. A schematic representation of the results of some of these methods is shown in Figure 2.25. The liver is the main source of most of these serum proteins. The parenchymal cells are responsible for the synthesis of albumin, fibrinogen, and other coagulation factors, and most of the α- and β-globulins. γ-Globulins are an important exception, being synthesized by B lymphocytes (91).

In this section, we only discuss proteins used in the diagnosis of liver disease: Albumin and prothrombin, both of which are synthesized exclusively by hepatocytes, and the Igs, which are synthesized by B lymphocytes. Additional diagnostic proteins include lipoproteins, ceruloplasmin, the blue copper-containing protein, ferritin, and α_1-antitrypsin.

Total serum protein is usually determined by a variation of the biuret reaction. Its fractionation into its major constituents, albumin and globulins, is performed with automated dye-binding methods, or with paper or cellulose acetate electrophoresis. Older, salting-out techniques do not remove all the α- and

β-globulins from albumin quantitatively, and are less commonly used.



• **Figure 2.25** Schematic representation of the electrophoretic pattern of normal human serum in pH 8.6 buffer obtained with four methods. **A:** Tiselius or free boundary electrophoresis. **B:** Paper electrophoresis. **C:** Starch-gel electrophoresis. **D:** Immunoelectrophoresis. *The arrow* indicates the starting point with each method. α_2-Macroglobulin remains at the origin in starch-gel electrophoresis, but moves in the γ- to β-range with other methods. Ig, immunoglobulin. (From Putnam FW. *The proteins*, 2nd ed., Vol. 1. Orlando: Academic Press, 1975:18, with permission.)

Albumin

Albumin, which is quantitatively the most important plasma protein, is synthesized exclusively by the liver. Normal serum values range from 3.5 to 4.5 g/dL (35 to 45 g/L). The average adult has approximately 300 to 500 g of albumin distributed in body fluids, and synthesizes approximately 15 g/day (200 mg/kg day). The synthesis rate may double in conditions in which rapid albumin loss or a decrease in serum albumin concentration occurs because of dilution, such as during the rapid accumulation of ascitic fluid (92). Albumin has a long half-life, approximately 20 days. Approximately 4% is degraded each day, but little is known about the site of degradation. The serum level at any time reflects the rate of synthesis and degradation, and the volume of distribution. Albumin synthesis is regulated by changes in nutritional status, osmotic pressure, systemic inflammation, and hormone levels (92,93). The precise mechanism is not entirely known, but appears to be related to the formation of albumin messenger ribonucleic acid (mRNA) polysomes within the liver (93). Substances that stimulate albumin synthesis cause individual ribosomes to bind to the albumin mRNA to form polysomes, which synthesize albumin more efficiently (93). Amino acids such as tryptophan, phenylalanine, glutamine, and lysine function in this way and increase albumin synthesis

in vitro (93). Albumin synthesis is also stimulated by the amino acids that increase urea synthesis, that is, ornithine and arginine (94). Ornithine serves as a precursor of the polyamine spermine, a compound that promotes polysome formation (94). Corticosteroids and thyroid hormone stimulate albumin synthesis by increasing the concentration of albumin mRNA and transfer RNA in hepatocytes either by increasing its synthesis or decreasing its degradation (95). In vitro, alcohol decreases albumin synthesis by inhibiting the formation of polysomes (93), whereas inflammation decreases albumin synthesis (96) through the inhibitory effects of interleukin-1 and tumor necrosis factor (97).

Serum albumin levels tend to be normal in patients with liver disease such as acute viral hepatitis, drug-related hepatoxicity, and obstructive jaundice. Albumin levels less than 3 g/dL associated with hepatitis should raise the suspicion of chronic hepatitis. Hypoalbuminemia is more common among individuals with chronic liver disorders, such as cirrhosis, and usually reflects severe liver damage and decreased albumin synthesis. One exception is patients with ascites, in whom synthesis may be normal or even increased, but serum levels are low because of the increased volume of distribution (92). Heavy alcohol ingestion, chronic inflammation, and protein malnutrition can inhibit albumin synthesis. Hypoalbuminemia is not specific for liver disease and may occur in protein malnutrition of any cause, such as protein-losing enteropathy, chronic infection, or nephrotic syndrome. Serum albumin should not be measured when screening patients with no suspicion of liver disease. A study of patients consecutively examined in a general medical clinic, showed that in 56 of 449 patients in whom no indications for albumin measurement were present, the results were abnormal. However, in only two patients (0.4%) was the finding of any clinical significance (98).

Prothrombin Time

Clotting is the end result of a complex series of enzymatic reactions that involve at least 13 factors. The liver is the major site of synthesis of 11 blood coagulation proteins:

Factor I—fibrinogen (99)

Factor II—prothrombin (100)

Factor V—proaccelerin, labile factor (100)

Factor VII—serum prothrombin conversion accelerator, stable factor (100)

Factor IX—plasma thromboplastin component, Christmas factor (100)

Factor X—Stuart-Prower factor (99)

Factors XII and XIII—prekallikrein and high-molecular-weight kininogen

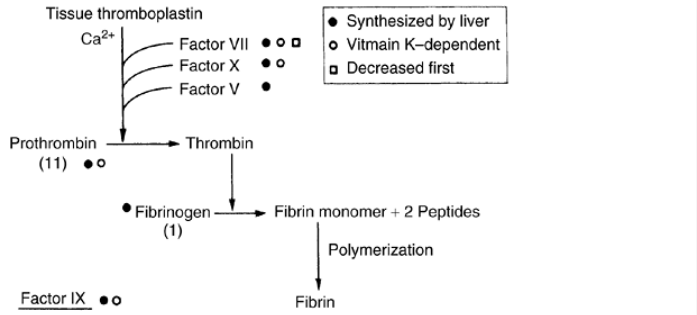
The liver is involved in clearing some of the clotting factors from serum. The levels of components of the clotting mechanism are frequently abnormal in the course of hepatic disease (101). These abnormalities can be assessed with tests in which one factor or the interplay of a number of factors is measured. The one-stage prothrombin time of Quick is one of the most useful tests available. It is used to measure the rate at which prothrombin is converted to thrombin. This occurs in the presence of a tissue extract (thromboplastin), calcium ions, and a series of activated coagulation factors (factors I, II, V, VII, and X), and is followed by the polymerization of fibrinogen to fibrin by thrombin (Fig. 2.26). The results may be expressed in seconds or as a ratio of the plasma prothrombin time to a control plasma time. A normal control is usually in the range of 9 to 11 seconds. A prolongation of 2 seconds or more is considered abnormal, and values of more than 4 seconds indicate a patient at risk of uncontrolled bleeding. The prothrombin time is prolonged if any of the involved factors are deficient, either singly, or in combination.

The international normalized ratio (INR) is often used to express the degree of anticoagulation in patients receiving warfarin sodium (Coumadin). The INR standardizes prothrombin time measurement according to the characteristics of the thromboplastin reagent used in the laboratory. The INR may not be the best expression of coagulation derangement in patients with liver failure, unless the same thromboplastin reagent is consistently used for measurement.

Hepatic synthesis of biologically active forms of factors II, VII, IX, and X requires vitamin K for the addition of carboxylic acid moieties to the g positions of glutamic acid residues in these proteins. The γ-carboxylation step is a posttranslational process that allows these proteins to bind Ca^{2+} avidly, a necessity for them to function as clotting factors (102). The absence of vitamin K, the ingestion of vitamin K antagonists, or the presence of certain hepatic disorders (hepatocellular carcinoma) inhibits vitamin K–dependent carboxylation and allows the release of des-γ-carboxy prothrombin (abnormal prothrombin) into serum (102). This can be detected with a specific radioimmunoassay (103). Healthy individuals have no abnormal prothrombin in serum. The presence of prothrombin in serum is a more sensitive indicator of vitamin K deficiency than measurement of prothrombin time, because an abnormal amount of prothrombin may be present in high concentration despite a normal prothrombin time. Prothrombin is present in high concentration in 91% of patients with biopsy-confirmed hepatocellular carcinoma (104). Plasma levels of des-γ-carboxy prothrombin do not correlate with α-fetoprotein levels in patients with established hepatocellular carcinoma,

but the two tests have a combined sensitivity of 85% (105). The des-γ-carboxy prothrombin level can return to normal with excision of or therapy for hepatocellular carcinoma, and increase again with a recurrence of the tumor (104). However, elevated des-γ-carboxy prothrombin levels were detected only in 20% of hepatocellular carcinomas less than 3 cm in diameter; therefore, the measurement of

this substance is an unsatisfactory screening test (106).



• **Figure 2.26** Factors involved in the Quick one-stage prothrombin time test.

A prolonged prothrombin time is not specific for liver disease and is seen in various congenital deficiencies of coagulation factors and in acquired conditions, including consumption of clotting factors and ingestion of drugs that affect the prothrombin complex. In these instances, the underlying cause can usually be elucidated. When the aforementioned conditions are excluded, a prolonged prothrombin time may be the consequence of either hypovitaminosis K, as is found in patients with prolonged obstructive jaundice, steatorrhea, dietary deficiency, or intake of antibiotics that alter the intestinal flora, or poor utilization of vitamin K owing to parenchymal liver disease. These two situations can usually be differentiated by means of parenteral administration of vitamin K_1. If the prothrombin time returns to normal or improves at least 30% within 24 hours of a single parenteral injection of vitamin K_1 (doses of 5 to 10 mg are usually given), it can be surmised that parenchymal function is good and that hypovitaminosis K was responsible for the original prothrombin time. In contrast, slight (if any) improvement occurs in most patients with parenchymal liver disease. Most patients with extrahepatic obstruction respond promptly to vitamin K. In patients with jaundice, the type of response to vitamin K_1 is therefore of some value in differential diagnosis. Observations of sluggish responses to vitamin K_1—prolonged values are still recorded 24 hours before normalization at 48 to 72 hours in some patients with obstructive jaundice—and of good responses among some patients with hepatocellular disease have been reported and complicate interpretation.

The prothrombin test is not a sensitive index of chronic liver disease because, even in severe cirrhosis, prothrombin levels can be normal or the prothrombin time only slightly prolonged. On the other hand, the test has high prognostic value, particularly for patients with acute hepatocellular disease. An abnormal prothrombin time with confirmed prolongation of more than 5 to 6 seconds above control is the single laboratory test that draws attention to the possibility of the development of fulminant hepatic necrosis in the course of acute viral hepatitis. Such a prolonged prothrombin time often precedes by days the manifestations of liver failure. Not all patients with abnormal prothrombin times of this extent have evidence of fulminant hepatic necrosis. Progressive shortening of the prothrombin time to normal usually precedes or accompanies other evidence of clinical improvement in the latter group. The degree of prolongation of the prothrombin time is a prognostic factor for patients with alcoholic steatonecrosis. A prothrombin time greater than 4 seconds above control value occurred six times as often in a group of patients who died (60%), than in a group who survived (10%). In patients with hepatocellular disease, an abnormal prothrombin time, particularly one prolonged more than 4 to 5 seconds that does not respond to parenteral administration of vitamin K_1, indicates extensive parenchymal damage and a poor long-term prognosis. The test is also used as an early predictor of outcome after acetaminophen overdose.

The prothrombin test is particularly important in the treatment of patients with liver disease. It allows assessment of the tendency to bleed before any contemplated surgical or diagnostic procedure,

such as closed liver biopsy, splenic puncture, or transhepatic cholangiography. When the prothrombin time is prolonged, vitamin K_1 should be administered routinely in doses of 5 to 10 mg/day parenterally up to three doses. It is difficult to identify the prothrombin time at which diagnostic procedures such as a needle biopsy of the liver are contraindicated, because the risk of bleeding has not been well correlated with the values of this test. Furthermore, vascular reactivity and coagulation factors, such as platelets, play an important contributory role. Closed needle biopsy is seldom performed in our hospital if the prothrombin time is prolonged more than 4 seconds. For patients with prothrombin times prolonged at least 4 seconds who do not respond to vitamin K_1, the mortality rate is high after surgery such as portacaval shunting. The performance of any open surgical procedure is determined by the urgency of the condition. The more pressing the need for surgery, the more prolonged is the prothrombin time accepted. It can often be corrected by infusions of fresh-frozen plasma with careful monitoring of the prothrombin time.

Tests Used to Detect Fibrosis in the Liver

There has been active research over the past decade to identify biochemical markers of liver fibrosis. The impetus for this research has been the desire to find a noninvasive alternative to liver biopsy. Liver biopsy remains the gold standard for grading and staging chronic liver disease. The single biochemical marker for fibrosis that has been studied most is hyaluronan. Others include type IV collagen, procollagen III, and laminin. Multiparameter tests have also been studied; these include the AST to platelet ratio index and the well-studied FibroTest biomarker, a multiparameter marker that includes haptoglobin, bilirubin, GGT, apolipoprotein A-I, and α_2-macroglobulin. Finally, there has been a recent report of glycomics being used to diagnose cirrhosis/advanced fibrosis.

Hyaluronan

Hyaluronan is a glucosaminoglycan synthesized in mesenchymal cells, and widely distributed in the extracellular space. It is degraded by hepatic sinusoidal cells in a specific receptor-mediated process, and has been found to be elevated in patients with cirrhosis because of sinusoidal capillarization. A fasting hyaluronan level greater than 100 mg/L had a 78% specificity and 83% sensitivity in the diagnosis of cirrhosis in one study of patients with liver disease of multiple etiologic factors (107). The sensitivity increased to 96% with a hyaluronan level greater than 300 mg/L. Hyaluronan has been shown useful for identifying advanced fibrosis in patients with alcoholic liver disease, hepatitis C, and hepatitis B (107,108,109). The serum hyaluronan level has been shown to be significantly correlated with the hepatic regeneration rate in patients undergoing hepatectomy (110), and with the development of liver failure after hepatectomy (111,112,113).

Fibrotest

The FibroTest has been studied in chronic hepatitis C, B, and alcoholic liver disease. In a study of patients with chronic hepatitis C, researchers defined "specific" and "sensitive" cutoffs for the activity index (114). The activity index is determined by a formula incorporating the five parameters listed in the preceding text. The specific cutoff had a sensitivity of 80% and specificity of 55% for identifying the presence of septal fibrosis (F2F3F4) or moderate or severe activity (A2A3) using the Metavir scoring system. The

sensitive cutoff had a sensitivity of 90% and specificity of 36%. A second group reported that the FibroTest performed poorly in predicting the presence or absence of significant liver fibrosis in patients with chronic hepatitis C (115). This biomarker produced similar results in hepatitis B (116) and alcoholic liver disease (117) as it did for hepatitis C. While the researchers who developed the FibroTest have reported low intralaboratory and intrapatient variability in FibroTest (118), a second group of researchers showed that intertechnique analytical variability of the FibroTest parameters remains a major issue (119). Specifically, depending on the assay used, there was a difference in values for apolipoprotein A-I of 12% and a 40% difference for α_2-macroglobulin.

Glycomics

Researchers applied the technique of using deoxyribonucleic acid sequencer-based total serum protein glycomics to generate profiles of serum protein *N*-glycans in patients with chronic liver disease (120). The authors chose to examine *N*-glycans because most glycoproteins found in the serum are produced by hepatocytes or plasma cells. Also, the liver is primarily responsible for the clearance of aberrantly glycosylated proteins. For these reasons it was felt that patients with chronic liver disease might have a different pattern of serum *N*-glycans, rather than control subjects. This technology yielded a biomarker that distinguished compensated cirrhosis from noncirrhotic chronic liver disease with a sensitivity of 79% and specificity of 86%. When combined with the FibroTest biomarker, the sensitivity was 75% with a sensitivity of 100%.

Tests Used to Detect Chronic Inflammation or Fibrosis in the Liver or Altered Immunoregulation

Immunoglobulins

Serum Igs are produced by stimulated B lymphocytes, and therefore, the measurement of these substances is not a direct test of liver function. Elevation of serum Ig levels in many patients with chronic liver disease is believed to indicate impaired function of reticuloendothelial cells in hepatic sinusoids or shunting of portal venous blood around the liver (91). Data indicate that antibodies directed against antigens of the normal colonic flora account for much of the increased serum Ig levels in patients with cirrhosis (91). In cirrhosis, these antigens are not taken up and degraded by hepatic reticuloendothelial cells as they normally are, but reach lymphoid tissue outside the liver where they elicit an inflammatory response.

In most cases of acute hepatitis, Ig levels are normal or minimally increased. Persistent moderate hypergammaglobulinemia suggests chronic active hepatitis, but striking increases in this Ig suggest autoimmune chronic hepatitis (121). Ig levels are also increased in most types of cirrhosis, although the values tend to be lower than in autoimmune chronic active hepatitis. Diffuse polyclonal increases in IgG and IgM are found in most types of cirrhosis and are nonspecific (121). Increases in IgM level suggest primary biliary cirrhosis, whereas increases in IgA may occur in patients with alcoholic cirrhosis. Ig levels are usually normal in obstructive jaundice. The major value of measuring Igs is to ascertain which patients might have cirrhosis or chronic hepatitis. It is also useful in monitoring the response to immunosuppressive therapy among patients with autoimmune chronic hepatitis. The presence of hypergammaglobulinemia, with or without hypoalbuminemia, is not specific for liver disease and can be found in other chronic inflammatory and malignant diseases.

Use of Liver Function Tests

We have found it useful to order the tests listed in Table 2.6 during the initial encounter with a patient with jaundice or suspected liver disease. The use of these tests, which include total and direct bilirubin, bilirubin in the urine, aminotransferases, alkaline phosphatase, albumin, globulin, and prothrombin time, as a battery increases specificity and sensitivity in the diagnosis of liver disease and makes it unlikely that any case of clinically important liver disease will not be identified.

It is helpful to divide the causes of jaundice and liver dysfunction into the broad categories shown in Table 2.6, that is, disorders of bilirubin metabolism, acute parenchymal or hepatocellular disease, cholestasis, infiltrative diseases, chronic hepatocellular diseases such as cirrhosis, and others. Because the pattern of LFT abnormalities is often similar in patients with infiltrative diseases and patients with partial bile duct obstruction, these two types of disorders are listed together.

In a patient with hemolysis or Gilbert's syndrome, the total serum bilirubin level may be elevated to as much as 5 mg/dL (85.5 mmol/L). If standard van den Bergh bilirubin fractionation is performed, more than 85% of the bilirubin is in the indirect fraction. Bilirubinuria is absent. If HPLC were used to fractionate the bilirubin, all the bilirubin would be in the indirect fraction. Results of the remaining screening LFTs, listed in Table 2.5, would be normal. Compensated hemolysis can be detected by means of measuring the reticulocyte count or the serum hemoglobin or haptoglobin. If bilirubinuria is present and more than 20% of the elevation in bilirubin level is due to the direct fraction, disorders of bilirubin metabolism, such as Dubin-Johnson syndrome and Rotor's syndrome, in which results of other LFTs are also normal must be considered. Because Dubin-Johnson syndrome and Rotor's syndrome are both benign conditions, it could be argued cogently that no further investigation is required, except to repeat these tests and verify the results. Those not satisfied with uncertainty can obtain a serum bile acid test, because the result is normal in Dubin-Johnson syndrome.

The aminotransferases are clearly the most sensitive agents for use in detecting acute hepatocellular disorders, such as viral or drug-induced hepatitis. Aminotransferase levels greater than 500 IU (8.3 μkat/L) make such a diagnosis probable. Depending on the severity of the underlying hepatocellular disorder, the bilirubin level may be normal or elevated. If the bilirubin level is elevated, bilirubinuria and increases in both the direct and the indirect bilirubin fraction should be present. Alkaline phosphatase elevations as high as three times the upper limit of normal are common for patients with acute hepatocellular disorders; however, values greater than three times normal are unusual, except in some patients with drug-induced hepatitis. Serum albumin level is usually normal, and Ig levels are normal or minimally elevated. The prothrombin time is typically normal in most patients with viral or drug-induced hepatitis. Prolongation of the prothrombin time to more than 5 seconds above the control and

failure of prothrombin time to correct within 24 to 48 hours of parenteral administration of vitamin K suggest a poor prognosis, and should alert the clinician to the possibility of massive hepatic necrosis. If the LFTs are typical of acute hepatitis, a careful medication history must be obtained along with serologic tests for hepatitis A and hepatitis B. The possibility of hepatic ischemia should also be considered.

Table 2.6. Liver Function Test Patterns in Hepatobiliary Disorders and Jaundice

Type of disorder	Bilirubin	Aminotransferases	Alkaline phosphatase	Albumin	Globulin	Prothrombin time
Hemolysis	Normal	Normal	Normal	Normal	Normal	Normal
Gilbert's syndrome	5 mg/dL >85% due to indirect					

	fraction					
	No bilirubinuria					
Acute hepatocellular necrosis (viral and drug hepatitis, hepatotoxins, acute heart failure)	Both fractions may be elevated; peak usually follows aminotransferases	Elevated, often >500 IU; ALT ≥ AST	Normal to three times normal	Normal	Normal	Usually normal; >5 s above control and not corrected with parenteral vitamin K suggests massive necrosis and poor prognosis
	Bilirubinuria					
Chronic hepatocellular disorders	Both fractions may be elevated	Elevated, but usually <300 IU	Normal to three times normal	Often decreased	Increased γ-globulin	Often prolonged; is not corrected with parenteral vitamin K
Alcoholic hepatitis	Bilirubinuria	AST/ALT >2 suggests alcoholic hepatitis or cirrhosis				
Cirrhosis						
Intrahepatic cholestasis	Both fractions may be elevated	Normal to moderate elevations; rarely >500 U	Elevated, often over four times normal	Normal, unless chronic	γ-Globulin normal	Normal; if prolonged, is corrected with parenteral vitamin K
Obstructive jaundice	Bilirubinuria				β-Globulin may be increased	
Infiltrative diseases (tumor, granuloma)	Usually normal	Normal to slightly elevated	Elevated, often over four times normal	Normal	Usually normal; γ-globulin may be increased in granulomatous disease	Normal
Partial bile duct obstruction			Fractionate, or confirm liver origin with 5′-nucleotidase, γ-glutamyl transpeptidase			

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Kaplan M. Evaluation of hepatobiliary diseases. In: Stein JH, ed. *Internal medicine*. Boston: Little, Brown, 1990:443, with permission.

LFTs are less precise in the diagnosis of chronic hepatocellular disorders, such as cirrhosis. The activity of the disease and the degree of hepatic reserve vary, and the results of LFTs vary with the activity of the disease process and the amount of hepatic reserve. LFTs may be highly insensitive in cirrhosis. Patients with end-stage postnecrotic or alcoholic cirrhosis may have shrunken livers and striking portal hypertension, and yet have LFT results that are almost normal. Breath tests, serum bile acid tests, and measurements of the undercarboxylated form of prothrombin would all be useful in such patients. On the other hand, LFT results may be strikingly abnormal in patients with chronic hepatic disorders. A serum albumin level of less than 3 g/dL, increased Ig levels, a prothrombin time 3 or more seconds above control that is not set right with parenteral vitamin K, and aminotransferase levels that are elevated but less than 300 IU make the diagnosis of cirrhosis likely. An AST/ALT ratio greater than 2 raises the possibility of alcoholic liver disease, and an AST/ALT ratio greater than 3 is highly suggestive of this possibility. Alkaline phosphatase level is seldom helpful in the diagnosis of cirrhosis.

Threefold or greater elevations should suggest the possibility of primary biliary cirrhosis in the setting of cirrhosis or portal hypertension.

There is a characteristic pattern of LFTs in cholestasis, although the routine laboratory tests listed in Table 2.6 do not differentiate intrahepatic and extrahepatic cholestasis. Alkaline phosphatase level is usually elevated out of proportion to the levels of other enzymes. Values four or more times greater than normal suggest some type of cholestasis. Depending on the severity of the underlying condition, the bilirubin level is either normal or elevated. If elevated, the direct fraction is increased and bilirubin is present. Aminotransferase levels are usually elevated, but values greater than 500 IU (8.3 μkat/L) are rare. Aminotransferase levels are usually less than 300 IU (5 μkat/L), unless the tests are performed within 24 hours of the development of acute bile duct obstruction due, for example, to the passage of a common duct stone. Albumin and globulin levels are usually normal. Increased Ig levels suggest cirrhosis in patients with cholestasis. An increased IgM fraction suggests primary biliary cirrhosis. The antimitochondrial antibody test is helpful in this situation. The result is positive in 90% to 95% of patients with primary biliary cirrhosis, and negative in patients with extrahepatic bile duct obstruction or sclerosing cholangitis. The prothrombin time is usually normal. If elevated, it is commonly due to vitamin K deficiency and should correct with parenteral administration of vitamin K.

Infiltrative liver diseases produce LFT abnormalities similar to those of partial bile duct obstruction. Often, the earliest and only abnormal value is alkaline phosphatase. If this is the case, or if the serum alkaline phosphatase level is elevated out of proportion to that of the other enzymes, it is helpful to identify the origin of the alkaline phosphatase. This can be done with electrophoretic techniques, or by means of measuring 5′-nucleotidase or GGT. The bilirubin level is often normal early in infiltrative disease, and is usually normal in partial bile duct obstruction. If the total serum bilirubin level is elevated, the direct fraction is certain to be elevated, and bilirubinuria is present. Aminotransferase levels are normal or minimally elevated in patients with infiltrative disease and partial bile duct obstruction, as are serum albumin and Ig levels and prothrombin time.

The results of these laboratory tests suggest but seldom confirm a specific diagnosis. Once this information is obtained, the results facilitate the efficient use of other diagnostic tests, such as the serologic testing for hepatitis, echography, computed tomography, percutaneous liver biopsy, and cholangiography.

With increasing experience, the breath and serum bile acid tests may be added to the list of routinely used tests in Table 2.6 or replace some of them. For example, serum bile acid tests may someday replace the direct bilirubin test as a general screening test for hepatic dysfunction. This has not yet occurred. Work continues on finding a test, or a combination of tests, that can eliminate the need for liver biopsy. Any conveniently performed inexpensive test that increases the diagnostic power of the standard LFT battery will be welcomed.

Footnote

[1]SI units (Le Système International d'Unites) are gradually replacing other units to have one common, worldwide system for reporting scientific data. The SI unit for enzymatic reactions is the katal, abbreviated kat. It replaces the international unit (IU/L) and denotes moles of substrate converted per second. For example, to convert alkaline phosphatase IU/L to SI units (μkat/L), multiply IU/L by 0.01667. The SI reference range for alkaline phosphatase is 0.5 to 2 μkat/L. This value may vary somewhat among laboratories.

Annotated References

Brensilver HL, Kaplan MM. Significance of elevated liver alkaline phosphatase in serum. *Gastroenterology* 1975;68:1556.

This paper describes the use of polyacrylamide gel electrophoresis to fractionate serum alkaline phos phatase in hundreds of patients with elevated serum alkaline phosphatase levels. The authors found that the elevation was caused by increased amounts of liver alkaline phosphatase in most patients and that patients with a variety of general medical disorders, such as congestive heart failure, Hodgkin's disease, and bacterial infection, could have a significant elevation in the level of serum alkaline phosphatase of hepatic origin.

Clermont RJ, Chalmers TC. The transaminase tests in liver disease. *Medicine* 1967;46:97.

The authors gathered all papers describing the evaluation of the AST (SGOT) test in the diagnosis of liver disease and determined the relation between the absolute value of the AST and the likelihood of a patient having obstructive liver disease, or, conversely, hepatocellular disease. The higher the AST level, the less likely for the patient to have obstructive

P.57

jaundice. Fewer than 1% of patients with obstructive jaundice have AST values greater than 700 IU.

Cohen JA, Kaplan MM. The SGOT/SGPT ratio: an indicator of alcoholic liver disease. *Dig Dis Sci* 1979;24:835.

The authors found that the SGOT/SGPT (AST/ALT) ratio is helpful in the diagnosis of alcoholic liver disease. If the ratio is greater than 2:1, alcoholic liver disease is likely. Ninety-five percent of patients with ratios greater than 3:1 have alcoholic liver disease.

Kaplan MM, Rhigetti A. Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct observation. *J Clin Invest* 1970;49:508.

This paper showed that the mechanism of the elevated serum alkaline phosphatase level in cholestasis is the induction of liver alkaline phosphatase and the leakage of liver alkaline phosphatase in serum. The authors clearly showed that the alternative theory popular at the time—that the liver clears alkaline phosphatase made in other organs from serum and excretes it into bile much as the liver excretes bilirubin—was incorrect. This paper resolved a controversy that had existed in medicine for more than 50 years.

Weiss JS, Gautam A, Lauff JJ, et al. The clinical importance of a protein bound fraction of serum bilirubin in patients with hyperbilirubinemia. *N Engl J Med* 1983;309:147.

The authors demonstrate that conjugated bilirubin in serum binds covalently to a macromolecule in serum (most likely albumin), and that the bilirubin fraction is cleared slowly from serum with a half-life similar to that of albumin. This finding demonstrated why it often takes so long for an elevated serum bilirubin level to decline in certain situations, such as when mechanical bile duct obstruction is surgically corrected.

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Chapter 3
Liver Biopsy and Laparoscopy

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Key Concepts

- Liver biopsy is the traditional gold standard for the evaluation of chronic liver diseases. However, sampling error, particularly evident with small specimens, makes biopsy a flawed benchmark.
- Percutaneous liver biopsy is a safe procedure both with or without radiologic guidance.
- Transjugular liver biopsies may be performed in patients with coagulopathies or other contraindications to percutaneous biopsy. Samples are often smaller and more fragmented.
- Diagnostic laparoscopy with liver biopsy is a low-risk, outpatient procedure performed under conscious sedation. Direct visualization of the liver surface improves the identification of cirrhosis and facilitates the staging of metastatic and primary cancers.
- Noninvasive panels of serologic markers are being studied to accurately grade and stage chronic liver disease, particularly hepatitis C. Transient elastography is a promising new technology for assessing hepatic fibrosis.
- The role of liver biopsy has been challenged in a number of clinical scenarios, including management of viral hepatitis, nonalcoholic fatty liver disease, monitoring of methotrexate hepatotoxicity, and in the diagnosis of hepatocellular carcinoma. In select cases, clinical, laboratory, and radiologic imaging data may obviate the need for a biopsy.

Introduction

Liver biopsy was first performed in 1883 by Paul Ehrlich (1). The procedure was lengthy and impractical until Menghini reported a quick "one-second" aspiration technique in the late 1950s (2). Transjugular hepatic vein catheterization in 1967 introduced a new biopsy technique with less risk for bleeding (3). Other technologic advances have expanded technical options for the performance of a liver biopsy. At present, large numbers of liver biopsies are performed safely each year, although life-threatening complications ensue occasionally. A variety of needles can be used during a percutaneous, transjugular, or laparoscopic approach. Both hepatologists and radiologists commonly perform biopsies either "blindly" or under radiologic guidance. Choice of technique depends on the

available expertise and the clinical situation. Percutaneous biopsy, with or without radiologic guidance, is most commonly performed and has a long track record of safety. The transjugular approach is often used when there are contraindications to percutaneous biopsy, such as a bleeding diathesis, obesity, or ascites. Laparoscopic liver biopsy is a more invasive procedure that is useful for the evaluation of various intra-abdominal malignancies and ascites of unknown cause. Fine-needle aspiration of focal liver lesions can be performed under radiologic guidance.

Liver biopsy has traditionally been the gold standard for the evaluation of chronic liver diseases. Recently, noninvasive markers of fibrosis and inflammation have been introduced, particularly for the evaluation of hepatitis C. As experience with these new techniques

grows, biopsy may be avoided in some clinical situations. Furthermore, with the advent of specific serologic testing, the need for liver biopsy has been challenged in some clinical scenarios. In hepatitis B and C, biopsy is useful, but not mandatory in making the decision to initiate treatment. Nonalcoholic fatty liver disease (NAFLD) can be diagnosed on the basis of clinical and imaging features, and biopsy generally does not influence management decisions. The role of periodic liver biopsy to assess for hepatotoxicity in patients taking methotrexate remains controversial. Finally, the role of liver biopsy in hepatocellular carcinoma (HCC) has become contentious because characteristic features on radiologic imaging, complemented by an elevated α-fetoprotein can now accurately diagnose these lesions.

Percutaneous Liver Biopsy

Before a percutaneous liver biopsy is performed, the patient must be prepared properly. A complete physical examination and history, review of medications, and measurement of clotting parameters are essential. Criteria for the safe performance of an outpatient biopsy and contraindications to a percutaneous approach are listed in Tables 3.1 and 3.2 (4). Explaining the procedure and possible minor and major complications and obtaining written consent from the patient are mandatory (5). It is preferred that prothrombin time, partial thromboplastin time, and platelet count be measured within 4 weeks of biopsy. Salicylates and nonsteroidal anti-inflammatory drugs are discontinued for 1 week before and 1 week after biopsy. A light breakfast 2 to 3 hours before the procedure can facilitate gallbladder emptying and may reduce the risk of gallbladder puncture. Alternatively, an overnight fast can be advised, particularly when conscious sedation is used, to reduce the risk of aspiration in case of vomiting. Venous access is established, preferably in the left arm, so that the patient can lie comfortably in the right lateral decubitus position after the biopsy. Patients are frequently anxious, and intravenous fentanyl and midazolam can alleviate apprehension, facilitate the procedure, provide some postprocedure relief of pain, and achieve some degree of amnesia. Most patients do well with approximately 50 μg of fentanyl and 2 mg of midazolam without impairment of their ability to cooperate with the biopsy. Older patients may require less sedation.

Table 3.1. Criteria for Outpatient Liver Biopsy

Patient must be able to return to the hospital in which the procedure was performed within 30 min of adverse symptoms
Patient must have a reliable individual staying with him or her during the first night after biopsy to provide care and transportation, if necessary
Patient should have no complications or associated serious medical problems that increase the risk of the biopsy
Facility in which the biopsy is performed should have an approved laboratory and blood banking unit, easy access to an inpatient bed, and personnel to monitor patient for at least 6 h after the biopsy
Patient should be hospitalized after the biopsy if any serious or persistent complications develop

Table 3.2. Contraindications to Percutaneous Liver Biopsy

ABSOLUTE
Uncooperative patient
Bleeding tendency
  Prothrombin time ≥4 s over control, international normalized ratio ≥1.5
  Platelet count <60,000/mm^3
Unavailability of blood transfusion support
Serious consideration of echinococcal disease
Presumed hemangioma or other vascular tumors
RELATIVE
Ascites
Infection in right pleural cavity
Infection below right diaphragm

The patient lies supine with the right hand placed under the head. The point of maximum liver dullness is percussed over the right hemithorax between the anterior and midaxillary lines and is usually found between the sixth and ninth intercostal spaces. In certain situations, it may be higher (e.g., obesity) or lower (e.g., emphysema). Dullness should be present in both inspiration and expiration, as some patients may inadvertently take a deep breath during the procedure and thereby risk a pneumothorax. One percent lidocaine (Xylocaine) is injected over the upper border of a rib, avoiding the intercostal nerve and vessels that run along the lower border. A small incision is made, and a suction needle containing saline solution is advanced through the subcutaneous tissue and peritoneum; during this process, two "pops" may be felt. A small amount of saline solution is flushed into the peritoneal cavity to expel any tissue that may have inadvertently entered the needle. After expiration, the patient holds his or her breath, and suction is applied to obtain a biopsy. A similar approach is used with cutting needles, either the manual Tru-Cut needle or the spring-loaded automatic needles.

Needles used to perform percutaneous liver biopsy are broadly categorized as suction needles (Menghini, Klatskin, and Jamshidi needles) and cutting needles

(Vim Silverman and Tru-Cut needles and the more recent spring-loaded needles with triggering mechanisms) (6). Different needles acquire various lengths

and diameters of hepatic tissue. Specimen diameters range from 1.0 to 2.0 mm. The automated devices generally acquire smaller samples. The cutting needles, except for those with a spring-loaded and triggering mechanism, generally remain in the liver for a longer time during the biopsy. A greater risk of bleeding after biopsy has been noted with larger-diameter needles, although not consistently (7,8). If cirrhosis is clinically suspected, a cutting needle is preferred to a suction needle because fibrotic tissue tends to be fragmented by the latter (9,10).

Most physicians perform an imaging study before biopsy to identify "silent" mass lesions and to define positions of the liver, gallbladder, and other organs. However, the routine use of ultrasonography to guide a percutaneous biopsy remains controversial. Currently in the United States, one half to three fourths of surveyed gastroenterologists who perform liver biopsy report the routine use of ultrasonographic guidance (11,12,13). In a prospective, randomized study of 836 patients, Lindor et al. (14) showed that ultrasonography with biopsy was less often associated with pain, compared to biopsies performed without radiologic imaging (37% vs. 50%). Furthermore, the postbiopsy hospitalization rate was lower if biopsy was performed with ultrasonography (0.5% vs. 2.2%); the most common reason for admission was pain (14). However, use of ultrasonography reduced neither the percentage of patients with pain requiring narcotics nor the rate of major complications (bleeding or hypotension) (14). Moreover, a large British survey failed to demonstrate differences in major complications or pain between biopsies performed with and without ultrasonographic guidance (15,16).

In contrast, a retrospective study using historical controls suggested that major complications may be reduced by the use of ultrasonographic guidance (17). In one study, ultrasonography changed the site of percutaneous liver biopsy determined by the percussion technique in as many as 15% of patients (18). If the site had not been moved, what complications may have resulted is not known. A follow-up study showed that ultrasonography may not be of any greater utility in selected patients, such as those with difficult percussion, obesity, or chest wall deformity. Ultrasonography was just as likely to mandate movement of the biopsy site in patients with these features compared to unselected patients (19). Cost-effectiveness studies have failed to resolve the controversy regarding the routine use of imaging to guide biopsy (20,21). A decision analysis suggested that the use of ultrasonography to prevent major complications was cost-effective, although the authors' definition of cost-effectiveness is not a standard one (cost-effective defined as incremental cost to avoid a major complication <$10,000) (20). Because of the established safety of the percussion technique and lack of convincing evidence of improved safety, tissue sample, or cost-effectiveness, routine ultrasonography to guide percutaneous biopsy should not be considered the standard of care (22,23) (Table 3.3).

Table 3.3. Ultrasonographic Guidance of Percutaneous Liver Biopsy

Advantages	Disadvantages
May decrease likelihood of	Has not been shown to decrease the

postbiopsy pain	frequency of major complications
May change biopsy site, as determined by percussion	Long track record of safety for performance without ultrasonographic guidance
May be cost-effective (controversial)	Adds expense to the procedure
	Inconvenient to perform

Because a liver biopsy samples at most 1/50,000th of the entire liver, it may not be representative. It is increasingly recognized that longer biopsy samples performed with larger bore needles are needed to accurately stage and grade the extent of liver injury. Traditionally, studies have accepted liver biopsies as adequate if samples were a minimum of 15-mm length, had 4 to 6 portal tracts, or were considered "acceptable" by a pathologist (samples as small as 10 mm have been used in some studies) (24). More recent data suggest that these minimum criteria are inadequate. Patients with Hepatitis C and biopsies of minimal length 1.5 cm showed significant variability in grade and stage between two simultaneous biopsies (at least 33% had a 1 point or more difference in stage) (25,26). In contrast, a study with mean specimen length of over 2.5 cm showed minimal variability between two samples taken from the same patient with hepatitis C (27). A recent study used digitized images of whole sections of hepatitis C–infected livers to allow for "virtual biopsies" of variable lengths. A 2.5-cm biopsy length was adequate to reliably stage fibrosis (28). Moreover, use of larger bore needles (>18 gauge) has also been shown to improve diagnostic accuracy (29). A study using large bore samples (1.4 mm) of at least 2 cm length reliably produced at least 11 to 15 complete portal tracts and allowed for accurate pathologic evaluation of chronic hepatitis B and C (30). In contrast, a recent study showed that even large biopsy samples may not be reliable for evaluation of NAFLD. Two biopsies with large bore (16 gauge) cutting needles of average length 2 cm were obtained in patients with NAFLD. There was surprising variability between the two biopsies with 41% discordance rate for one or more stages. These results could only partially be explained by intraobserver variability (31). We can therefore conclude that larger bore biopsies of longer length are desirable to reliably grade and stage chronic

liver disease (Table 3.4). However, liver biopsy must be regarded as a flawed gold standard for the evaluation of chronic liver diseases because of significant problems with sampling variability.

Table 3.4. Characteristics of an Optimal Biopsy

≥2–2.5 cm long

≥1 mm diameter (use at least 18-gauge needle) ≥11–15 complete portal tracts

Complications of Percutaneous Liver Biopsy

Although the liver is a highly vascular organ, major complications associated with this procedure are fortunately rare (Table 3.5). The most dreaded complication is hemorrhage. Sixty percent of complications are recognized within 2 hours after the procedure, and 96% within 24 hours (5,7). Fatal complications typically occur within 6 hours. A hospitalization rate of 1.4% to 3.2% for the management of complications following a liver biopsy has been reported, with pain or hypotension being the predominant cause (32,33).

Approximately one third of patients experience right upper quadrant or right shoulder pain (16), although one study reported a rate of up to 83% (34). The pain is usually mild, dull, and responsive to analgesics. Pain medications are required in approximately one third of patients after biopsy, approximately one half of whom are given narcotics (35). While pain typically resolves within a few hours of biopsy, one group found that it may persist up to 24 hours after biopsy (34). On-going severe abdominal pain should alert the physician to the possibility of bleeding or peritonitis. Predictors of pain include a history of intravenous drug abuse and preprocedural anxiety (34,35). Prebiopsy anxiolysis with a benzodiazepine like midazolam might be expected to prevent or lessen postbiopsy pain, although this has not been formally studied.

Table 3.5. Complications of Percutaneous Liver Biopsy

Pain (0.056%–83%)
  Pleuritic
  Peritoneal
  Diaphragmatic
Hemorrhage
  Intraperitoneal (0.03%–0.7%)
  Intrahepatic[a] and/or subcapsular (0.59%–23%)
  Hemobilia (0.059%–0.2%)
Bile peritonitis (0.03%–0.22%)
Bacteremia
Sepsis (0.088%) and abscess formation

Pneumothorax and/or pleural effusion (0.08%–0.28%) Hemothorax (0.18%–0.49%) Arteriovenous fistula (5.4%)[b] Subcutaneous emphysema (0.014%) Reaction to anesthetic (0.029%) Breaking of needle (0.02%–0.059%) Biopsy of other organs Lung (0.001%–0.014%) Gallbladder (0.034%–0.117%) Kidney (0.029%–0.096%) Colon (0.0038%–0.044%) Mortality (0.0088%–0.3%)

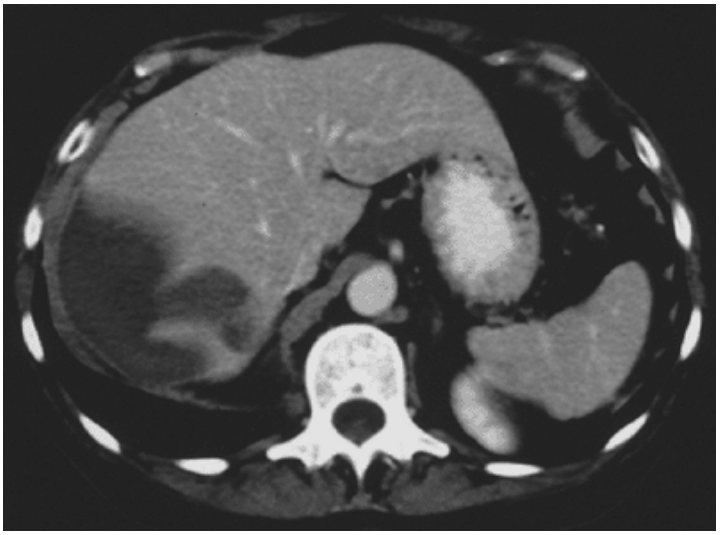
[a]Symptomatic and asymptomatic.
[b]Asymptomatic.
From references (4,7,16,33,34,39–41,52).

Hemorrhage after liver biopsy can manifest as a free intraperitoneal bleed, intrahepatic or subcapsular hematoma, or hemobilia. Risk factors for hemorrhage after liver biopsy include presence of malignancy, performance of multiple passes, advanced age, mycobacterial infection, requirement for prebiopsy platelet transfusion, acute liver failure, cirrhosis, heparin administration on the day of biopsy, treatment with corticosteroids, and use of nonsteroidal anti-inflammatory agents (7,8,36). Significant intraperitoneal hemorrhage is the most serious complication and often becomes apparent within the first 2 to 3 hours after the procedure (7,8,33). Free intraperitoneal hemorrhage may be related to a laceration sustained during deep inspiration or to a penetrating injury of a large vessel. In a study of 68,276 liver biopsies, hemoperitoneum occurred in 0.32% of patients (7). Patients may experience abdominal pain, tachycardia, and hypotension, and ultrasonography or computed tomography (CT) scan may confirm the clinical suspicion. Volume resuscitation must be undertaken immediately. If hemodynamic instability persists despite aggressive resuscitation over a couple of hours, then angiography with embolization of the bleeding site is the preferred approach (37,38). In some cases, surgical exploration is required.

Intrahepatic or subcapsular hematomas are noted in up to 23% of cases when ultrasonography is performed after a liver biopsy (39,40,41). These are usually asymptomatic, and the frequency is similar in both laparoscopically guided and "blind" liver biopsies (41). The duration of bed rest after a liver biopsy does not influence the likelihood of hematoma formation (39). Large hematomas may cause pain, liver enlargement, tachycardia, hypotension, and a delayed drop in the hematocrit (Fig. 3.1). Conservative treatment generally suffices, and angiography is rarely required.

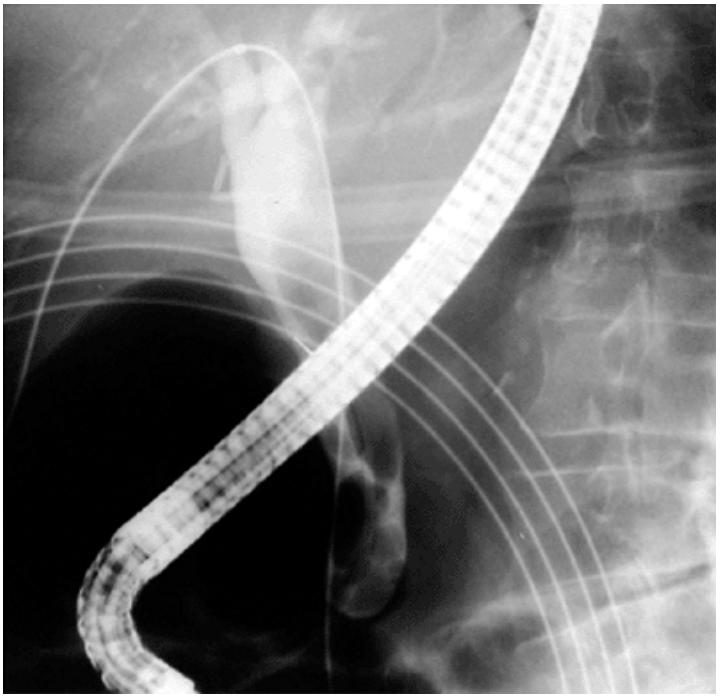
Hemobilia is an infrequent complication of liver biopsy, presenting with the classic triad of gastrointestinal bleeding, biliary colic, and jaundice (42). Piccinino et al. (7) reported four cases out of 68,276 biopsies (7). Bleeding is usually arterial in origin, but can be venous. Its severity ranges from life-threatening hemorrhage to occult bleeding with chronic anemia. In contrast to intraperitoneal hemorrhage, hemobilia is

generally delayed, with mean onset of 5 days after biopsy (42,43).



▪ **Figure 3.1** Computed tomography scan demonstrating an intrahepatic and subcapsular irregular, hypodense collection in the liver, consistent with bleeding after a percutaneous liver biopsy.

Endoscopy may reveal blood flowing from the ampulla of Vater. Endoscopic retrograde cholangiopancreatography (ERCP) demonstrates linear, irregular densities in the biliary tree and gallbladder that do not have the configuration of gallstones (Fig. 3.2), and endoscopic sphincterotomy may facilitate the removal of these clots. However, angiography is the preferred modality for diagnosis and embolization therapy.



▪ **Figure 3.2** Endoscopic retrograde cholangiopancreatographic finding of irregular filling defects in the common bile duct, representing blood clots after hemobilia complicating a percutaneous liver biopsy. (Radiograph courtesy of Gregory Ginsberg, MD)

Transient bacteremia has been reported in 5.8% to 13.5% of patients (44,45) after liver biopsy. This is usually of no clinical significance, although sepsis can sometimes occur in patients with biliary obstruction (46,47,48). No recommendations are currently available regarding prophylactic antibiotics for those with risk factors for endocarditis. Other complications of liver biopsy include asymptomatic intrahepatic arteriovenous fistula, biliary ascites, bile pleuritis, and biliary peritonitis (49,50,51,52). The most common cause of bile peritonitis is puncture of the gallbladder, which becomes apparent immediately and is characterized by severe pain and vasovagal hypotension. Bile peritonitis is also more likely if biliary obstruction is present. Pain may be followed by fever, abdominal pain, leukocytosis, and ileus. Ultrasonography or CT scan may identify an intra-abdominal collection of bile, and ERCP or biliary scintigraphy may demonstrate a bile leak. Surgery is indicated when clinical deterioration occurs despite the administration of intravenous antibiotics, fluid, and pain control. Pneumothorax and hemothorax after liver biopsy often spontaneously resolve, and placement of a chest tube is seldom required. Inadvertent perforation of other abdominal organs is generally not of clinical significance (7).

Transjugular Liver Biopsy

During transjugular biopsy, liver tissue is obtained from within the vascular system to minimize the risk of bleeding. The right internal jugular vein is punctured percutaneously, a catheter is passed under fluoroscopy into the right hepatic vein, and a liver specimen is obtained with a special needle. Electrocardiographic monitoring is performed to detect arrythmias associated with the passage of the wire through the heart. Infrequently, it may be necessary to perform the biopsy from the left internal jugular vein after attempts to enter from the right side have failed. A femoral vein approach has also been described (53,54).

Adequate tissue can be obtained in 80% to 100% of cases (55,56,57,58,59,60,61). In a single large experience involving 1,000 transjugular liver biopsies, the success rate was as high as 99.3%, with one or two attempts required in 57.7% of cases (56). Multiple passes are usually required to obtain adequate samples, and typical length of the tissue ranges from 0.3 to 2.0 cm. Biopsies obtained by the transjugular approach tend to be smaller, more fragmented, and include fewer complete portal tracts, as compared to tissue acquired by the percutaneous route (57,59). Aspiration needles and manual and automated Tru-Cut–type needles with spring-loaded devices are available (55,62,63,64). Cutting needles provide larger transjugular biopsy samples than those obtained with aspiration devices (63,64).

Table 3.6. Situations in which Transjugular Biopsy is Preferred over a Percutaneous Approach

Coagulopathy (international normalized ratio >1.5)
Thrombocytopenia (platelets <60,000/μL)
Ascites
Obesity
Fulminant liver failure
Vascular hepatic tumor
Peliosis hepatis

The procedure is indicated for patients with significant coagulopathy, moderate to severe ascites, or fulminant hepatic failure (Table 3.6). Other indications include a previously failed percutaneous liver biopsy, massive obesity, and suspected vascular tumor or peliosis hepatis. A transjugular biopsy is advisable if concomitant measurement of the hepatic venous pressure gradient or placement of transjugular intrahepatic portosystemic shunt is planned.

Major complications after transjugular liver biopsy include major hemorrhage with hepatic capsule perforation and bacterial cholangitis after fistula formation between the biliary tree and the portal or hepatic vein. These complications occur at a rate of 1.3% to 2.7% with mortality of less than 0.3% (57,60,62). Minor complications may include pain, bleeding from the neck puncture site, neck hematoma, cardiac arrhythmia, and pneumothorax.

Liver Biopsy in Special Patient Populations

Patients with clotting parameter abnormalities or thrombocytopenia require special consideration before planning a liver biopsy. Transjugular liver biopsy is generally preferred for patients with a platelet count of less than 60,000/μL or prothrombin time prolonged by 4 seconds or more (international normalized ratio [INR] >1.5). A study has observed that patients with mild thrombocytopenia (50,000 to 99,000/μL) or prothrombin times prolonged by less than 4.2 seconds do not have increased risk of bleeding after percutaneous liver biopsy (65). Such patients may not benefit from prebiopsy platelet transfusion or fresh frozen plasma administration. The use of prophylactic fresh frozen plasma for a prothrombin time of 4.0 seconds or more (INR ≥1.5) with rechecking of the prothrombin time after infusion has been recommended and widely adopted. However, data supporting this practice are not available (66). In a recent retrospective study among patients with severe thrombocytopenia and hematologic malignancy, transjugular liver biopsy was safely performed after platelet transfusion to a median posttransfusion platelet count of 30,000/μL. The authors propose 30,000/μL as a threshold for platelet transfusion before performing the transjugular liver biopsy (67). Until better safety data are available, we recommend the transjugular approach with prophylactic platelet transfusion for patients with platelet count less than 60,000 μL. For patients with either thrombocytopenia or increased prothrombin time, the use of fibrin glue may prevent bleeding. In this patient population, percutaneous biopsy followed by injection of fibrin glue to seal the tract has been performed with good safety

(68,69). We do not recommend the routine use of bleeding time to assess the risk of bleeding after liver biopsy. Bleeding time measurement is controversial and is unlikely to provide much value (70,71).

Patients with chronic renal failure may have deficient platelet functioning despite a normal platelet count. Dialysis performed the day before the procedure or the use of deamino-8-D-arginine vasopressin (DDAVP) just before the procedure has been advocated, but should not be regarded as mandatory. A recent study of patients with chronic renal failure did show a significantly lower rate of major hemorrhage after transjugular liver biopsy, as compared to percutaneous puncture (72).

Patients taking an oral anticoagulant should discontinue the drug at least 72 hours before liver biopsy, provided discontinuation is not contraindicated. Heparin may be started approximately 24 hours after the biopsy. Immediate restarting of oral anticoagulation after biopsy is not preferred because delayed bleeding has been reported in patients taking oral anticoagulants (73,74). It is preferred that oral anticoagulation be restarted 48 to 72 hours after biopsy.

Aledort et al. (75) reported a rate of significant bleeding as high as 12.5% among hemophiliacs who underwent liver biopsy before 1981 (75). This high bleeding rate prompted serious concerns regarding biopsy in this population, but other studies have not reproduced those results. Reports of percutaneous liver biopsies performed in hemophiliacs during short hospitalizations with clotting factor replacement have shown no significant bleeding (76,77,78,79,80,81). Case series of transjugular liver biopsies performed in hemophiliacs after clotting factor infusions have also demonstrated excellent safety (82,83,84,85).

Laparoscopy

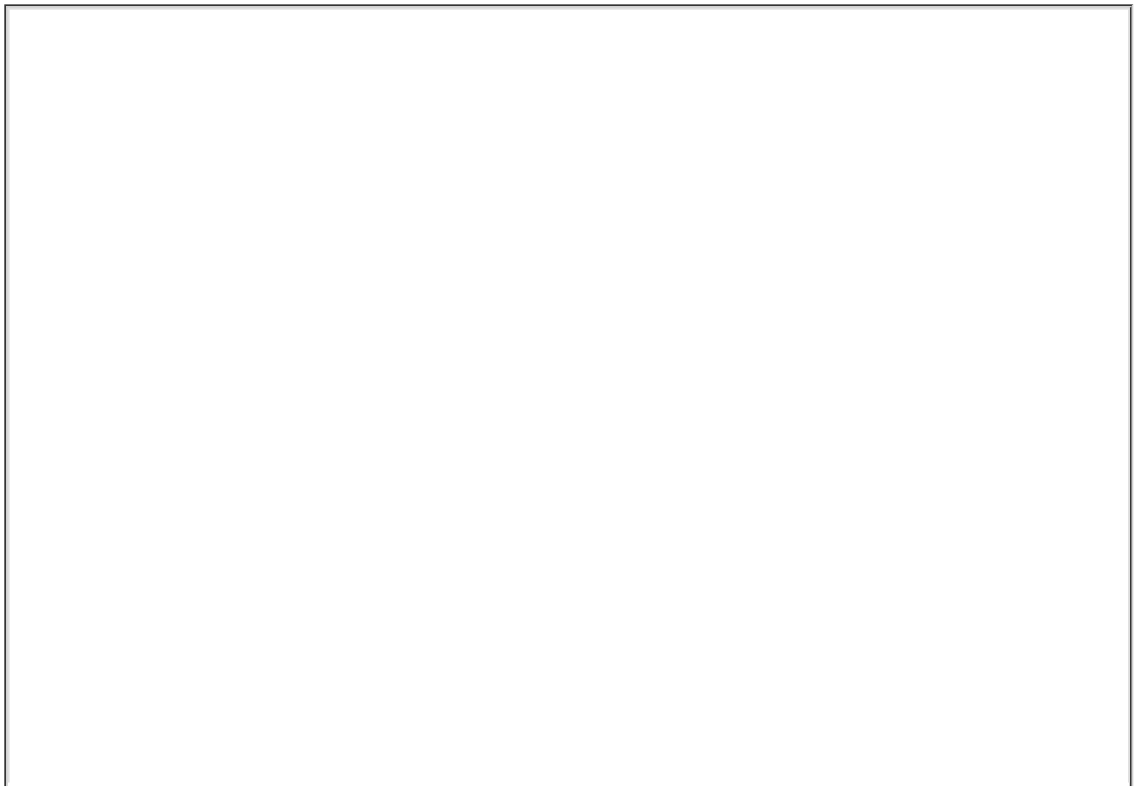
Use of laparoscopy to evaluate liver disease is well established in Continental Europe, but is much less frequently performed in North America than in other parts of the world. During laparoscopy, direct inspection of the liver surface is performed and tissue is acquired. The procedure can be performed under conscious

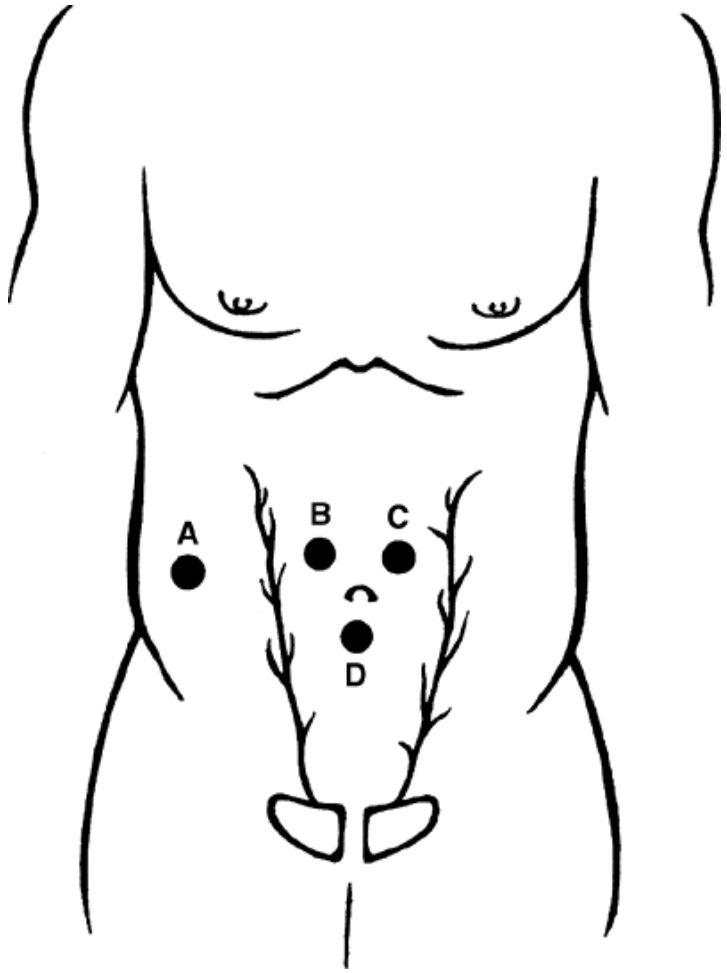
sedation in the outpatient setting. Recent introduction of mini-laparoscopy and laparoscopic ultrasonography has made the procedure potentially safer and more attractive.

Technique

Although laparoscopy is commonly performed under general anesthesia, the safety and efficacy of outpatient diagnostic laparoscopy using conscious sedation has been demonstrated (86,87,88). In some centers, diagnostic laparoscopy is performed in the endoscopy suite. With the patient in a supine position, the abdomen is scrubbed with povidone-iodine (Betadine) and covered with sterile drapes. Application of 2-L oxygen by nasal cannula and monitoring with electrocardiography and pulse oximetry are recommended (89,90). The Veress needle and trocar are usually placed in the left paramedian area; however, a right paramedian or subumbilical approach can be used in patients with an enlarged left hepatic lobe, splenomegaly, or previous splenectomy (Fig. 3.3). A local anesthetic (1% lidocaine) is injected intradermally 2 cm above and to the left of the umbilicus (Fig. 3.4A). Then, a 16-gauge needle is inserted through the center of the wheal to the parietal peritoneum, which usually provokes some

pain. Approximately 15 to 20 mL of 1% lidocaine is applied to the subcutaneous tissue and fascia within a 2-cm radius. It is important that sufficient local anesthesia be applied. A small incision is made in the center of the wheal, and the patient is asked to distend the abdominal wall without arching the back. The Veress needle is then inserted into the abdominal cavity, and two distinct "pops" are heard (91). Aspiration with a 10-mL syringe may avoid air embolism or inadvertent entry into the intestines, both of which are rare complications (92,93). Whereas carbon dioxide used for insufflation during therapeutic laparoscopy is a peritoneal irritant and provokes pain, the nitrous oxide commonly used for diagnostic laparoscopy is better tolerated (89,90). Insufflation to an abdominal cavity pressure of 20 mm Hg is accomplished by delivering 3 to 6 L of nitrous oxide through the Veress needle (Fig. 3.4B). A 20-mL syringe, half filled with saline solution, is then inserted and rotated within the abdominal cavity. Gas bubbles within the syringe indicate an unobstructed area for trocar placement. The patient is then instructed to distend the abdomen, and the trocar is inserted into the peritoneal cavity. Two distinct "pops" confirm placement. An oblique-view laparoscope is then inserted into the abdominal cavity under direct vision. The area perpendicular to the scope is inspected for insertion-related damage. With the patient in Trendelenburg's position, the bladder and other pelvic structures can be visualized. Placement of the patient in reverse Trendelenburg's position allows thorough inspection of the right and left upper quadrants (94). A second trocar is inserted into the right midclavicular line to allow, via another laparoscope, the inspection of the superior aspect of the right lobe and the delivery of accessory equipment, such as the biopsy needle and palpating probe. Liver specimens are obtained with a biopsy gun or, less commonly, a Tru-Cut needle. To avoid large blood vessels, a tangential approach to the liver left of the falciform ligament is recommended (Fig. 3.4C,D,&E). More recently, mini-laparoscopy has been described with use of a 1.9-mm end-viewing instrument (95,96,97). A randomized study between mini- and conventional laparoscopy demonstrated similar success with both procedures, and shorter procedure time with mini-laparoscopy (97).

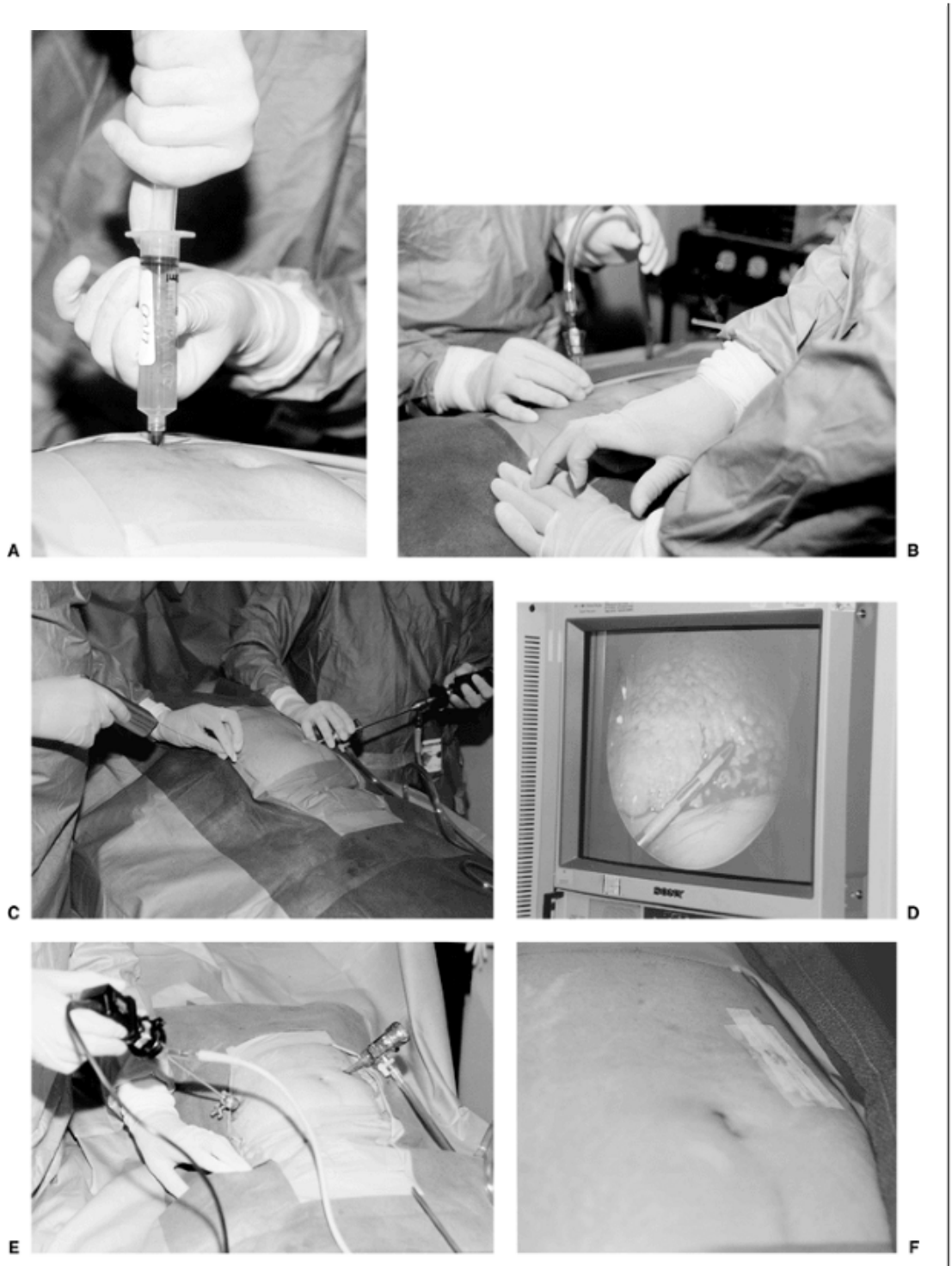




▪ **Figure 3.3** Veress needle and trocar entry sites. *A*, Secondary trocar site for the visualization of the superior aspects of right lobe and placement of accessory equipment. *B*, Alternate site in patients with splenomegaly or previous left upper quadrant surgery. *C*, Preferable site with excellent view of left lobe. *D*, Subumbilical alternate site, least vascular.

After the examination is completed, the trocar and biopsy sites can be closed with Steri-Strips or by sutures if larger incision is made to accommodate larger trocars and laparoscopes (Fig. 3.4F). Patients are observed for approximately 18 to 24 hours postprocedure and discharged to resume regular activity in 3 to 4 days. Right shoulder pain for 6 to 8 hours after the procedure is common. In a survey of 215 patients with gastrointestinal malignancies who underwent outpatient

staging laparoscopy under conscious sedation, only 7% required narcotics 2 to 5 hours after the procedure (87).



▪ **Figure 3.4 A:** Laparoscopic site with injection of local anesthetic to the left of the umbilicus. **B:** Nitrous oxide placed through Veress needle with assistant verifying the loss of hepatic dullness. **C:** Laparoscopic guidance of biopsy gun. **D:** Palpation probe on biopsy site after procedure to ensure hemostasis. **E:** Three-millimeter mini-laparoscope visualizing the original laparoscopic trocar site for signs of bleeding. **F:** Completion of procedure; laparoscopic site closed with Steri-Strips.

Patients should avoid nonsteroidal anti-inflammatory drugs and salicylate

compounds for 1 week before and after the procedure. Recommendations for patients with clotting factor and platelet count abnormalities are similar to those of percutaneous liver biopsy. In patients with end-stage renal disease, laparoscopy can be performed safely the day after dialysis with careful observation of the biopsy site before the procedure is terminated (98). Recently, the use of recombinant factor VIIa before laparoscopic liver biopsy has been reported (99). Moreover, mini-laparoscopy appeared safe in a small study of 61 patients with a platelet count less than 50,000/μL and/or INR greater than 1.5 (100). Most patients required the application of argon plasma coagulation directly to the liver to stop postbiopsy bleeding; there were no reports of delayed bleeding (100).

Indications for Laparoscopy

Laparoscopy allows direct visualization of the liver and can be performed for a variety of indications (Table 3.7). Although reporting of laparoscopic findings has not been rigorously standardized, chronic liver disease causes a spectrum of changes in the gross appearance of the liver (Fig. 3.5). In patients with mild disease, the surface of the liver tends to appear smooth. With more advanced disease, granularity or early nodularity may be seen. Cirrhosis is associated with diffuse nodularity and features of portal hypertension. Liver biopsy specimens tend to understage the degree of fibrosis assessed by laparoscopic inspection. Liver biopsy, while specific for cirrhosis, may be up to 30% less sensitive for demonstrating cirrhosis as compared to laparoscopy (95,96,97,101,102). Furthermore, specific laparoscopic features, including the presence of irregular regenerative nodules, a high degree of nodular regeneration, and an atrophic right lobe, may predict the development of HCC in patients with hepatitis C cirrhosis (103).

Table 3.7. Indications for Diagnostic Laparoscopy

Chronic liver disease
Hepatocellular carcinoma
Benign hepatic tumors
Staging of gastrointestinal malignancy
Metastatic liver disease
Ascites of unclear cause
Peritoneal infection
Lymphoma
Fever of unknown origin
Evaluation of abdominal mass
Chronic abdominal pain
Hepatosplenomegaly of unclear cause
Liver disease in renal failure
Assessment for liver transplantation
Subfulminant hepatic failure

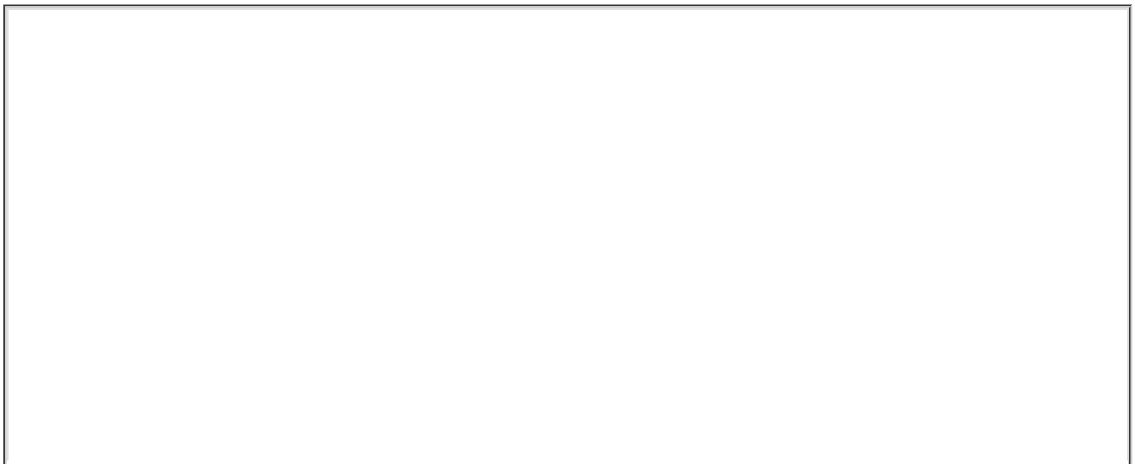
Diagnostic laparoscopy can help in evaluating ascites of unknown cause. During laparoscopy, tuberculous peritonitis commonly appears as miliary lesions in the parietal peritoneum and occasionally in the liver. Thick adhesions of the bowel to the parietal peritoneum may be seen (104). In one series, tuberculous peritonitis

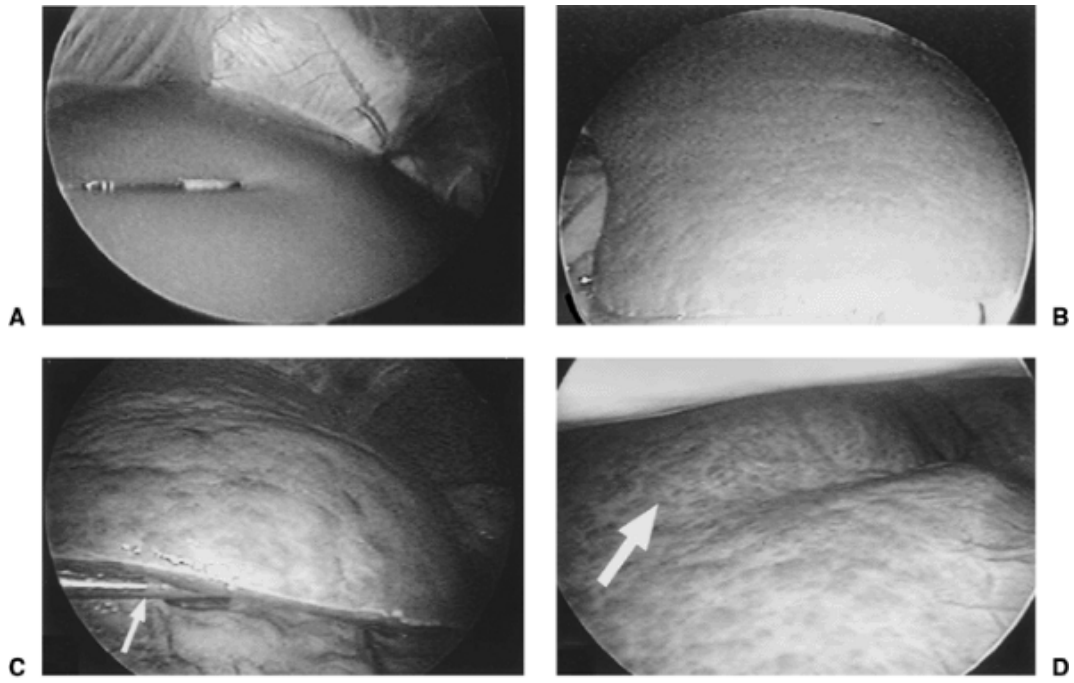
was associated with ascites in only 60% of cases (105). Chu et al. (106) performed 129 laparoscopies for ascites of unclear cause (106). They reported peritoneal carcinomatosis in 61%, tuberculous peritonitis in 20%, a nondiagnostic procedure or miscellaneous causes in 14%, and cirrhosis in 5% of cases. Laparoscopy may also identify lymphoma in patients with human immunodeficiency virus (HIV) and ascites (107).

Laparoscopy is used both diagnostically and therapeutically in the management of HCC. The liver surface may demonstrate changes suggestive of HCC, such as hypervascular nodules and hyperemic, pigmented lesions (108). The risk of sampling HCC is controversial, but most cases of biopsy-proven seeding of the needle track have been reported after percutaneous biopsy (109, 110). Laparoscopy can also be used in patients with suspected HCC to assess the extent of the primary lesion and to examine other areas for synchronous tumors (111). In addition, it may be helpful to perform laparoscopy in patients with rising levels of α-fetoprotein and unrevealing imaging studies. Furthermore, laparoscopy with ultrasonography is superior in the diagnosis of HCC compared to laparoscopy alone (112). Local therapy with ethanol injection, microwave coagulation, or radiofrequency ablation has been applied laparoscopically or percutaneously; nevertheless, recurrence of HCC at the port site has been described after local therapy (113, 114).

Laparoscopy is effective for staging a variety of cancers. In a study of pancreatic cancer, laparoscopy identified metastases undetected by CT scan in 31% of patients (115) (Fig. 3.6). Other studies have validated the usefulness of laparoscopy to help identify pancreatic cancer patients who could benefit the most from surgical resection (115,116,117,118,119). In a decision analysis model comparing multiple strategies for the staging and treatment of pancreatic cancer in which CT scan was performed first, endoscopic ultrasonography followed by laparoscopy was shown to minimize costs and unnecessary surgical explorations (120). Laparoscopic staging is also valuable in identifying surgical candidates for the management of cancers of the esophagus, stomach, ampulla, and bile ducts (121). Finally, laparoscopy is a valuable aid in staging lymphoproliferative diseases and requires the conversion to an open procedure in less than 5% of cases (122,123,124). In one

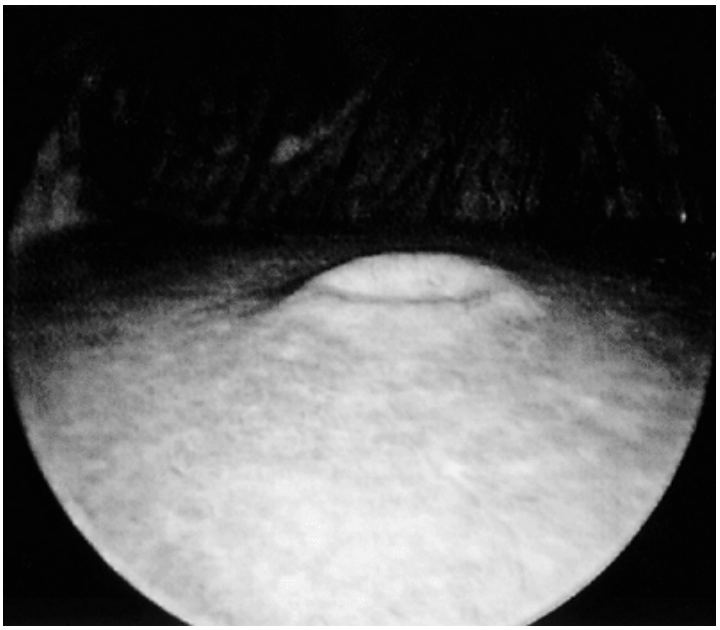
study, laparoscopy was more sensitive than CT scan for the detection of lymphomatous invasion of the liver. White spots or nodules visualized during laparoscopy were 100% specific for lymphoma (125). As imaging technologies continue to evolve, the added value to performing staging laparoscopy must be assessed on an on-going basis.





▪ **Figure 3.5** Progression of hepatitis C in four different stages. **A:** Smooth appearance of liver, stage I histology. **B:** Finely granular appearance of liver, stage II. **C:** Uneven surface with early nodularity (early cirrhosis). **D:** Nodular liver with large regenerating nodule in the superior aspect of the right lobe.

Introduction of laparoscopic ultrasonography has further improved the accuracy of staging gastrointestinal malignancy (126). During laparoscopic ultrasonography, the liver parenchyma is swept with a linear array probe placed in contact with the liver surface. The operator can also obtain tissue samples and perform radiofrequency ablation. General anesthesia is commonly used. The value of laparoscopic ultrasonography has been demonstrated in a number of settings. In a prospective study, laparoscopic ultrasonography prevented unnecessary laparotomy in 65% of patients referred for the resection of HCC (127). Moreover, laparoscopic ultrasonography changed surgical decision making in 36% of patients referred for the staging of pancreatic carcinoma; 5% of those patients had appeared unresectable by conventional imaging (128). Laparoscopic ultrasound may also play a role in staging tumors of the proximal bile duct and gallbladder (129).



▪ **Figure 3.6** Staging laparoscopy in a patient with pancreatic carcinoma; 8-mm lesion with crater seen in the left lobe.

Complications of Laparoscopy

In a large series of 1,794 diagnostic laparoscopies, major complications occurred in only 8 patients (0.44%), minor complications were observed in 31 patients (1.73%), and one death occurred (93) (Table 3.8). Minor complications, such as vasovagal reactions, subcutaneous emphysema, pneumoperitoneum, and abdominal pain, can be controlled during the procedure (93,130,131,132,133). Bleeding from the biopsy site is controlled with the application of lateral pressure, use of a heater or bicap probe, or the topical application of thrombin. Delayed bleeding or hemobilia is seen primarily in patients with portal hypertension. Bowel perforation can be a risk in patients with a history of multiple surgeries, bacterial peritonitis, or tuberculous

peritonitis (93,106). If the trocar site is found to be bleeding, a second trocar can be inserted to visualize the bleeding source. Avitene plugs placed within the trocar track are generally effective in stopping bleeding.

Table 3.8. Complications of Diagnostic Laparoscopy in 1,794 Patients at the University of Miami (93)

Complication	No. (%)
MAJOR	
Abdominal viscus perforation	3 (0.16)

Bleeding from liver biopsy site	2 (0.11)
Hemobilia	2 (0.05)
Spleen laceration	1 (0.05)
Total	8 (0.37)
MINOR	
Ascitic fluid leakage	9 (0.50)
Abdominal wall hematoma	6 (0.33)
Fever	6 (0.33)
Vasovagal reaction	5 (0.27)
Prolonged abdominal pain	4 (0.22)
Seizures	1 (0.05)
Total	31 (1.70)

Noninvasive Surrogates for Biopsy

Although liver biopsy is generally considered the gold standard for the evaluation of liver disease, sampling errors may limit its accuracy. In contrast, noninvasive markers may potentially represent a more global assessment of liver injury. Furthermore, noninvasive methods may be safer, more attractive to patients, and cheaper than biopsy. Three main types of surrogates for biopsy have been investigated: Indirect markers (aspartate aminotransferase [AST] to alanine aminotransferase [ALT] ratio, aspartate aminotransferase to platelet ratio index [APRI], FibroTest, and ActiTest), direct markers of extracellular matrix turnover (hyaluronic acid and YKL-40), and ultrasonographic evaluation of liver stiffness (FibroScan) (Table 3.9). Most studies have focused on the evaluation of the extent of fibrosis and degree of inflammation in hepatitis C. Noninvasive identification of significant fibrosis (often defined as portal fibrosis with some septae) allows stratifying patients with hepatitis C most in need of therapy.

Table 3.9. Noninvasive Surrogates for Biopsy

Test	Description	Comments
INDIRECT MARKERS		
AST/ALT ratio	Simple index derived from widely available blood tests	Ratio >1 has limited accuracy in predicting cirrhosis in viral hepatitis and NAFLD
APRI	Ratio of AST/upper limit normal to platelet count × 100	Good prediction of significant fibrosis in hepatitis C (APRI <0.5 and >1.5 predict absence and presence, respectively) Good prediction of cirrhosis in hepatitis C (APRI <1.0 and >2.0 predict absence and presence, respectively)
FibroTest	Commercially available panel of α_2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, bilirubin	Good prediction of significant fibrosis and cirrhosis in hepatitis C and B
ActiTest	Commercially available panel which consists of all elements of FibroTest and includes ALT	Reliable prediction of moderate activity in hepatitis C and B
DIRECT MARKERS		
Hyaluronic acid	Marker for extracellular matrix turnover	Very sensitive for cirrhosis in viral hepatitis and alcoholic liver disease. Low value rules out cirrhosis
YKL-40	Marker for extracellular matrix turnover	Some utility to predict fibrosis
OTHER		

FibroScan	Transient elastography Probe induces vibration and ultrasonographically measures transmission through liver to estimate stiffness (fibrosis)	Excellent test for fibrosis and cirrhosis in hepatitis C. Has not been evaluated in populations with significant obesity

AST, aspartate aminotransferase; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; NAFLD, nonalcoholic fatty liver disease; GGT, γ-glutamyl transferase.

Receiver operator characteristics (ROC) curve analysis is an important method to compare the test characteristics of noninvasive markers. ROC curves plot the sensitivity and specificity achieved for different test thresholds. Excellent diagnostic tests are those that achieve good sensitivities and specificities over a wide range of possible test "cutoff" values. Area under the receiver operator characteristics curve (AUROC) greater than 0.9 is excellent and that between 0.8 and 0.9 indicates a good test. Noninvasive markers cannot be expected to achieve perfect test characteristics because these panels are compared to liver biopsy,

a modality that does not perfectly assess global liver injury. When only large biopsies are used as the gold standard, noninvasive markers have demonstrated better test characteristics (134,135).

Simple, widely available, noninvasive tests have some value in predicting cirrhosis. Ultrasonography is widely used in the evaluation of liver diseases. It is moderately specific for identifying cirrhosis, but not sensitive (136). An increased AST/ALT ratio greater than 1 helps identify cirrhosis in viral hepatitis and NAFLD, particularly if the increased ratio is associated with a platelet count of less than 130,000/μL (137,138,139). Furthermore, APRI has demonstrated excellent ability to predict cirrhosis (AUROC 0.89 to 0.94) and good ability to predict significant fibrosis (AUROC 0.80 to 0.88) in patients with hepatitis C (140). A recent study in patients with hepatitis C confirmed good test characteristics for APRI, but observed that the AST/ALT ratio was a poor test (AUROC = 0.57 for significant fibrosis and AUROC = 0.73 to 0.75 for cirrhosis) (141).

The MULTIVIRC group studied 339 patients using a panel of five nonroutine serum markers (α_2-macroglobulin, haptoglobin, apolipoprotein A-I, γ-glutamyl transferase, and total bilirubin) in patients with hepatitis C. This panel of markers, now known as *FibroTest*, demonstrated an AUROC between 0.83 and 0.85 for the prediction of significant fibrosis. Fifty percent of patients could be accurately stratified using the test and could thereby avoid a liver biopsy (142). Subsequent studies have validated these findings in other people with hepatitis C (134,143,144,145). A somewhat less robust prediction has been demonstrated in chronic hepatitis B (146). Moderate or severe activity in hepatitis C can be predicted accurately by the ActiTest, which uses the same set of markers as FibroTest and also includes ALT (134). In hepatitis B, reliable prediction of moderate or severe activity was achieved with the ActiTest (AUROC = 0.82), which was similar to the prediction simply by AST or ALT tests (AUROC = 0.82,

0.81, respectively) (146). Forns et al. (147) developed another panel of markers (age, platelet count, γ-glutyl peptidase, and cholesterol) with good ability to predict significant fibrosis in hepatitis C (147).

Direct markers of extracellular matrix formation and removal have demonstrated less clinical utility to date. Hyaluronic acid is very sensitive (97%) for cirrhosis in viral hepatitis and alcoholic liver disease, and a low level can be used to rule out cirrhosis (148,149). Different test thresholds must be used for different etiologies of liver disease (148). YKL-40 is a glycoprotein involved in tissue remodeling and has also shown some utility as a predictor of fibrosis (149). Recently, a combination of hyaluronic acid, tissue inhibitor of metalloprotease-1, and α_2-macroglobulin was successfully used to identify significant fibrosis in hepatitis C (AUROC = 0.83) (150).

Transient elastography (FibroScan) is a promising new technique to evaluate fibrosis. A probe applied to the abdomen transmits a low-frequency vibration. The vibration induces an elastic shear wave, which is propagated faster in stiffer (more fibrotic) liver tissue. Ultrasonographic interrogation of the wave allows measuring the shear wave velocity. Tissue elasticity can then be determined. FibroScan has demonstrated good prediction of significant fibrosis and excellent prediction of cirrhosis (135,151). A potential concern with the technique is that it has so far been studied only among French patients with relatively low body mass indices. How successful FibroScan will be in higher body mass index (BMI) populations, such as in the United States, is not yet known.

A recent study compared noninvasive assessment of fibrosis in patients with hepatitis C using APRI, FibroTest, and FibroScan (145). All three tests achieved similar AUROC for the evaluation of significant fibrosis and cirrhosis. The authors suggest that the use of FibroTest and FibroScan together achieves the most accurate assessment of fibrosis. However, it is not obvious that the diagnostic combination is much better than performing one test alone. Cost-effectiveness studies are needed to help clarify the role of these new technologies.

Indications for Liver Biopsy

Liver biopsy is often an important component of the evaluation of chronic liver disease. With advances made in serologic and imaging tests, its use in some situations has been challenged. We describe in the subsequent text some disease states in which the routine use of liver biopsy has been questioned (Table 3.10).

Hepatitis C

Chronic hepatitis C infection globally affects approximately 170 million individuals. Candidates for treatment need to be carefully selected because hepatitis C therapy is associated with significant side effects, considerable expense, and less-than-ideal response rates, particularly in genotype 1 patients. Furthermore, most infections are asymptomatic and not progressive (152).

Liver biopsy aids in identifying treatment candidates because it is currently the best predictor of progressive disease. On the basis of retrospective data, most patients with moderate inflammation develop cirrhosis within 20 years and nearly all patients with severe inflammation or bridging fibrosis develop cirrhosis within 10 years. Patients with mild inflammation and/or minimal fibrosis have a low risk of progression to cirrhosis (153). Hepatic steatosis is also emerging as a major risk factor for fibrosis progression in hepatitis C (154,155).

As experience with FibroTest, FibroScan, APRI, and other noninvasive markers grows, fewer biopsies will likely need to be performed to evaluate the urgency of initiating hepatitis C treatment.

Table 3.10. Utility of Biopsy in Specific Clinical Situations

Issue	Pros of biopsy	Cons of biopsy
HEPATITIS C		
Prognosis	Extent of fibrosis and inflammation are best predictors of disease progression	Noninvasive markers may accurately stage and grade disease
Decision to treat	Genotype 1: Identify those most in need of therapy (therapy longer in duration and less likely to succeed)	Genotypes 2 and 3: Patients motivated for therapy may forgo biopsy (therapy shorter in duration and more likely to succeed)
Treatment-related side effects	Severity of liver disease helps in deciding whether to endure or stop therapy	Commitment to therapy should be independent of disease severity
Previously treated	Lower success with retreatment Identify those most in need of therapy (advanced fibrosis)	Motivated patients who are genotype 2 or 3, were previously treated with interferon monotherapy, had significant dose reductions, or were on-treatment responders have relatively good prospects for response to retreatment
HEPATITIS B		
Decision to treat	Consider biopsy if minimal elevation or fluctuating ALT; consider treatment if at least moderate inflammation	Hepatitis B serologies, hepatitis B virus DNA, and ALT generally determine decision to treat

Identify cirrhosis	Prompts screening for varices and HCC	HCC surveillance recommended whether cirrhosis is present or not
ABNORMAL HEPATIC BIOCHEMICAL TESTS AND NAFLD		
Elevated ALT	Confirm diagnosis	Cause accurately identified clinically in >90% cases without biopsy
Diagnosis of NAFLD	Patients may not have classic NAFLD risk factors	Accurate diagnosis of NAFLD generally possible without biopsy
Identify severity of NAFLD	Only biopsy can distinguish simple steatosis from steatohepatitis	Noninvasive markers may be developed to distinguish the two
Treatment of NAFLD	Presence of steatohepatitis or fibrosis may motivate some to undertake risk factor modification	There is no proven therapy for NAFLD Absence of steatohepatitis or fibrosis may remove motivation for some to undertake risk factor modification

ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease.

Liver biopsy may also reveal unsuspected cirrhosis, which would prompt initiation of a surveillance strategy for HCC and esophageal varices. Presence of advanced fibrosis is also negatively associated with the probability of response to hepatitis C therapy. In a study of pegylated interferon-α 2b with ribavirin, the absence of bridging fibrosis or cirrhosis was significantly associated with a sustained virologic response (57% vs. 44%) (156).

Clinical information may help refine prognosis but cannot substitute for the valuable data obtained from biopsy. Risk factors for accelerated fibrosis progression include age at infection greater than 40 years, daily alcohol consumption, male gender, ALT, increased BMI, immunosuppression, and coinfection with hepatitis B or HIV (157,158,159,160,161,162,163,164). Twenty-five percent to 40% of patients with chronic hepatitis C have persistently normal ALT levels (165,166). This group tends to exhibit less fibrosis and inflammation than patients with hepatitis C and elevated ALTs (167). Moreover, in one study of 102 patients, the median progression of fibrosis was twice as fast in an elevated ALT group compared to a persistently normal ALT group (168). Despite these

reassuring findings, patients with persistently normal ALTs may have significant inflammation (19% with moderate inflammation) (169) and even cirrhosis (6%) (170).

Although the information obtained from liver biopsy is useful, it is not mandatory to perform biopsy before treating hepatitis C. Patients with genotype 2 or 3 may undergo therapy regardless of findings on liver biopsy because therapy is likely to succeed (80%) and its duration is short (24 weeks). Biopsy is more useful for genotype 1 patients. Because therapy is long in duration (48 weeks) and less likely to succeed (<50%), only patients at risk for progressive liver disease may opt for therapy. Once treatment is started, knowing the extent of fibrosis, inflammation, and steatosis may help patients and providers decide whether to continue therapy in the face of significant side effects. Furthermore, patients undergoing treatment with pegylated interferon and ribavirin can expect a low chance of sustained virologic response if they failed prior interferon monotherapy (28%) or prior interferon and

ribavirin therapy (12%) (171). Therefore, patients with mild disease should not generally attempt retreatment. Recent studies have shown that in the absence of therapy, few patients progress by more than one stage of fibrosis over 3 to 5 years (158,159,160). Therefore, repeating liver biopsy after 3 to 5 years is reasonable to reassess the need for treatment on an on-going basis. In select cases, biopsy may be avoided before initiating retreatment. Motivated patients who are genotype 2 or 3, and were prior on-treatment responders, had significant dose reductions, or were previously treated with only interferon monotherapy have relatively good chances for response to retreatment.

Although histologic improvement may be demonstrated after successful hepatitis C treatment, a posttreatment biopsy is generally not useful. In nonresponders, the role that liver biopsy should play in identifying candidates for maintenance therapy is still unclear. In one study of virologic nonresponders who had achieved a histologic response after 6 months of therapy, patients were randomized to 2 years of maintenance interferon or placebo. Treatment led to a decrease in fibrosis (172) We await data from the on-going Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial of maintenance pegylated interferon for patients with advanced fibrosis (171).

Hepatitis B

Liver biopsy is frequently performed in hepatitis B, but clinical and serologic information are more often used to determine its management. Biopsy can identify unsuspected cirrhosis, which would prompt a surveillance strategy for esophageal varices. Screening for HCC should be considered in all hepatitis B surface antigen–positive patients, even in the absence of cirrhosis. Risks for HCC include acquisition of virus in an endemic area, male gender, family history of HCC, cirrhosis, and coinfection with hepatitis C or D (173,174). Risk factors for progressive liver disease include older age, persistence of serum hepatitis B viral DNA, occurrence of acute serologic exacerbations, elevated ALT, and presence of severe inflammation on liver biopsy (175,176,177). A recent study showed that both ActiTest and ALT correlate well with histologic inflammation (146).

Ideal candidates for treatment of chronic hepatitis B have significant hepatitis B viremia ($\geq 10^5$ copies/mL if hepatitis B e-antigen positive and $\geq 10^4$ copies/mL if hepatitis B e-antigen negative). To expect successful therapy, serum transaminases are ideally elevated at least two times above the upper limit of

normal (178,179,180,181,182). Histologic findings generally do not affect the decision of whether to initiate treatment, although there are some important exceptions. Patients with cirrhosis and hepatitis B virus viremia should undergo treatment (173,174). Furthermore, it may be reasonable to perform liver biopsy in patients with fluctuating or minimal elevation in transaminases (<2 times the upper limit of normal). A small percentage of these patients will have severe inflammation and, if treated, have an acceptable chance of response (183). Liver biopsies performed on patients with viremia and with normal ALTs (immune-tolerant phase) show no or mild liver disease (184). Such patients do not require treatment until ALT is consistently elevated, and biopsy is not recommended (174). Generally, treatment length is determined by serologic endpoints and not by changes demonstrated on histology from repeated biopsy.

Nonalcoholic Fatty Liver Disease and Abnormal Hepatic Biochemical Tests

Hepatologists are commonly asked to evaluate patients with chronically elevated hepatic biochemical tests. Determining the etiology requires a careful history, physical examination, review of medications and alcohol use, serologic studies, and imaging. Generally, an etiology can be identified without liver biopsy. In most of the remaining cases, the diagnosis is NAFLD. For example, in one series of 1,124 patients presenting for evaluation of chronically elevated hepatic biochemical tests, only 81 patients (8%) had an undetermined etiology after complete clinical evaluation. On liver biopsy, 73 patients had NAFLD, and 8 biopsies were normal (185). Sorbi et al. (186) reported on 36 patients with persistently elevated hepatic biochemical tests and an unrevealing noninvasive investigation. Prebiopsy diagnosis was NAFLD in 67%. Biopsy led to a change in diagnosis in only 14%, 80% of whom had normal liver biopsies (186). In contrast, one study observed that biopsy directly affected management in 18% of patients with persistently abnormal hepatic biochemical tests (187).

Liver biopsy is considered the gold standard for the diagnosis of NAFLD. However, clinical suspicion with supportive radiologic imaging and absence of serologic findings are adequate to diagnose NAFLD in most patients without incurring the expense, inconvenience, and risk of liver biopsy. Metabolic syndrome (obesity, dyslipidemia, and/or diabetes mellitus) is present in most patients with NAFLD, although some investigators have found significant numbers of patients without these characteristic risk factors (188,189). Radiologic imaging can complement clinical suspicion for diagnosing NAFLD. Ultrasound, CT scan, and magnetic resonance imaging (MRI) are highly accurate for identifying at least moderate steatosis; however, imaging cannot distinguish steatohepatitis or fibrosis from simple steatosis (190,191).

Histologic findings of steatonecrosis and fibrosis identify a subset of patients with a worse prognosis (192,193). Cirrhosis is present in 10% to 20% of patients and moderate to severe fibrosis, in up to 40% (189,194,195). Clinical features are helpful, but not definitive, for prognosis. Risk factors for worse disease include age over 45, obesity, diabetes mellitus, hypertriglyceridemia, hypertension, and increased ALT (139,194,196). Recent reports have highlighted the observation that the entire histologic spectrum of nonalcoholic steatohepatitis (NASH) can exist among patients with normal ALT (197,198).

The major benefit of performing liver biopsy is to distinguish patients with steatosis from those with steatohepatitis. Certainly this distinction is critical to

studies of investigational agents for the treatment of NASH. Apart from studies, current therapy for NASH focuses on addressing obesity, diabetes mellitus, dyslipidemia, and hypertension. No specific therapy can yet be recommended for NAFLD. Performing a biopsy may motivate some patients to pursue treatment of metabolic syndrome more aggressively. On the other hand, if a biopsy shows only mild changes from NAFLD, some patients with metabolic syndrome may derive a sense of false security that they need not make major changes in diet and exercise. We generally do not perform biopsy to evaluate NAFLD.

Methotrexate-Induced Hepatotoxicity

Methotrexate is a potentially hepatotoxic drug used to treat psoriasis, rheumatoid arthritis, and other diseases. Evidence-based recommendations for monitoring liver toxicity differ between the dermatology and rheumatology literature. Early studies reported that up to 26% of patients with psoriasis treated with long-term methotrexate developed cirrhosis (199). Dermatology guidelines recommend pretherapy liver biopsy in high-risk patients, followed by serial biopsy after every cumulative exposure of 1 to 1.5 g of drug. Demonstration of moderate fibrosis would preclude further therapy (200). A more recent series using modern fibrosis-scoring methods reported a much lower risk of methotrexate injury, and the authors raise questions about the necessity for such intensive monitoring (201).

Reports of significant fibrosis and cirrhosis are relatively rare among patients with rheumatoid arthritis taking methotrexate. Lower incidence of hepatotoxicity may be due to more rigorous exclusion of chronic liver disease in the rheumatology literature or to a disease-specific risk associated with methotrexate hepatic injury. Because persistent AST elevations have been associated with liver injury (202), rheumatology guidelines recommend biopsy only if most ASTs during the course of a year are elevated. An elevated AST prompts reduction in methotrexate dose, and treatment is discontinued if a biopsy demonstrates moderate fibrosis. Pretreatment biopsy is performed if chronic liver disease is suspected (203). Long-term safety has been demonstrated in patients with rheumatoid arthritis adhering to this monitoring schedule (204). Our approach to potential methotrexate-induced hepatotoxicity in patients with both rheumatoid arthritis and psoriasis is to perform a pretreatment liver biopsy in selected patients who have risk factors for chronic liver disease and for those with persistent elevations of aminotransferases during therapy.

Hepatocellular Carcinoma

HCC must be suspected in a patient with cirrhosis and a focal liver lesion. Sensitivity of fine-needle biopsy for the detection of HCC is between 86% and 90% and depends on nodule location and size (more often diagnostic for lesions >3 cm) (205,206,207). Noninvasive methods of diagnosing HCC include tumor markers and radiologic studies. Although α-fetoprotein may not be elevated in up to 40% of HCC, a significant elevation can help make the diagnosis. In one study, an α-fetoprotein greater than 200 ng/mL had 99% specificity for HCC (208). CT scan and MRI have demonstrated excellent accuracy for diagnosing HCC (209). Recent European guidelines suggest that HCC can be diagnosed without biopsy if a characteristic mass greater than 2 cm in a cirrhotic liver is evident in two imaging modalities. Elevated α-fetoprotein (>400 ng/mL) may help confirm the diagnosis (210).

Biopsy of HCC carries a significant risk of needle-track seeding (1.6% to 5%)

(109,205,206). A recent report of a polyethylene shield device may reduce that risk, but is as yet unproven (211). If a diagnosis of HCC can be confidently made on the basis of imaging with or without the help of α-protein, we do not perform fine-needle liver biopsy to confirm the diagnosis. At our institution, biopsy of HCC is generally reserved for patients in whom no definitive surgical intervention is planned and can be obtained at the time of nonsurgical treatment (radiofrequency ablation, alcohol ablation, or chemoembolization).

Conclusion

Liver biopsy is a time-honored and safe method to evaluate chronic liver diseases. Growing evidence suggests that biopsies may not be representative of disease processes affecting the entire liver, especially when small samples are obtained. Biopsy is generally performed percutaneously with or without radiologic guidance. Transjugular biopsies may be performed on

patients with ascites, obesity, or a bleeding diathesis. Laparoscopy, more commonly used in Continental Europe, is a safe outpatient procedure useful for accurately determining the presence of cirrhosis and for staging various gastrointestinal cancers.

Recently, panels of markers that can serve as surrogates to biopsy in identifying the extent of fibrosis and inflammation have been introduced. As experience grows with these tests, it is possible that performance of biopsy may decline in some clinical situations, such as identifying candidates for hepatitis C therapy. Routine use of biopsy has been questioned in a number of clinical scenarios. In hepatitis C, biopsy may help stratify which patients are at risk for progressive disease and therefore most in need of therapy. Genotype 2 or 3 patients have a high response rate and a shortened treatment course and may therefore opt for therapy without biopsy. In hepatitis B, biopsy generally does not affect treatment decisions except in the subset of patients with minimally elevated or fluctuating transaminase levels. Distinction of NASH from bland steatosis can only be accomplished by biopsy. In the absence of any specific therapy for patients with either NASH or NAFLD, biopsy results currently do not affect management decisions. In monitoring methotrexate toxicity, the need for periodic liver biopsy has been questioned, and most patients can avoid biopsy if transaminases remain relatively normal. Finally, radiologic imaging can now accurately diagnose HCC without risking possible needle-track seeding from biopsy.

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Chapter 4

Imaging

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Key Concepts

- Recent technologic advances have led to considerable improvements in liver imaging.
- Ultrasonography is a quick, widely available, and inexpensive modality for hepatic imaging and is often the initial imaging test ordered.
- Multidetector computed tomography (CT) scan and magnetic resonance imaging (MRI) allows three-dimensional imaging of the hepatic parenchyma and vasculature.
- Evaluation of hepatic malignancies is facilitated by 2-fluoro-deoxy-D-glucose (FDG) positron emission tomography.
- Ultrasonography, noncontrast CT scan, and magnetic resonance imaging (MRI) are able to demonstrate the presence of hepatic steatosis; however, none of these imaging modalities is able to distinguish between steatosis and steatohepatitis.
- Ultrasonography with Doppler imaging is a useful first tool for the diagnosis of hepatic venous thrombosis; MRI allows superior visualization of the inferior vena cava and hepatic veins, as well as liver parenchyma.
- Ultrasound guidance is commonly used for percutaneous liver biopsies in patients with diffuse liver disease. Although studies have suggested that this practice may result in fewer complications, more data are needed before this can be established as a uniform standard.

Noninvasive Imaging of the Liver

Recent technological advances have allowed considerable improvement in imaging of the liver. This introductory section provides a short description of the imaging modalities, with brief guidelines on which test to use and when. An in depth explanation of the physics is beyond the scope of this text. The aim is that the referring physician will understand the advantages and limitations of the imaging modalities available. It is hoped that this will facilitate the choice of the most appropriate diagnostic tool for better and more cost-effective patient care. The second portion of the chapter will address selected clinical problems in which critical use of radiologic modalities plays a role in evaluation and management.

Ultrasound

This test should serve as a baseline for nearly all patients who initially present with symptoms suggestive of liver or gallbladder disease. It is fast, cost-effective, noninvasive, widely available, and involves no radiation.

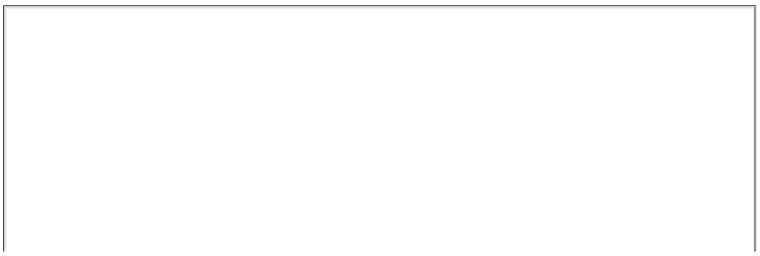
The basic principle of ultrasound is the transmission of sound waves into the liver from a transducer. These waves reflect at interfaces between tissues with differing acoustic impedance. For example, at the soft tissue–air interface almost total reflection occurs, which explains the use of ultrasound gel to allow the sound waves to pass into the body. As the ultrasound wave passes through the liver, the sound wave is affected by changes in tissue type and is refracted and reflected. The transducer is able to detect the reflected sound waves and uses the time delay from transmission to

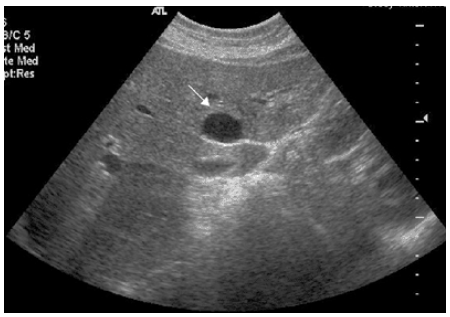
calculate depth within the body. The ultrasound image displays the amplitude of reflection as a function of position, with the "whiteness" of the image directly proportional to the amplitude of reflection. The liver is best evaluated with a 2 to 5 MHz ultrasound transducer. In larger patients, the lower frequency permits greater tissue penetration. The higher frequency transducers offer improved spatial resolution but less penetration. Therefore, imaging of superficial structures should be preferentially performed with a higher frequency transducer.

The appearance of a lesion within the liver is explained by its relative reflectivity compared with the surrounding liver parenchyma. When the sound wave passes through a structure without any reflection, no echoes are generated and the structure appears black or anechoic, such as a hepatic cyst (Fig. 4.1). When nearly all of the sound wave is reflected, the structure appears white or hyperechoic such that the lesion appears brighter than the liver. This is well demonstrated with a cavernous hemangioma whose classic appearance is that of a homogenously hyperechoic structure (Fig. 4.2). This is secondary to its complex internal structure whose network of vascular structures almost completely reflects the ultrasound beam. When some of the sound waves pass through, and some are reflected, the lesion will be gray or hypoechoic. This means that the lesion is less bright than the liver. An example is colorectal metastases (Fig. 4.3).

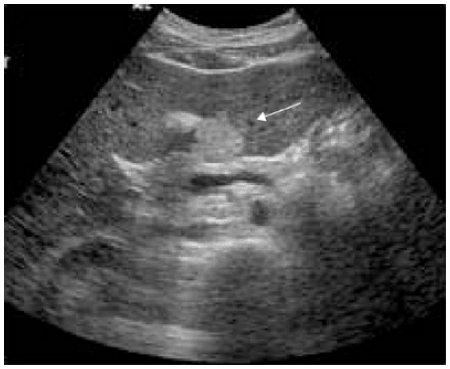
Tissue harmonic imaging is an alternative method of producing an image. When an ultrasound wave insonates tissues in the body, this produces secondary sound waves at integral multiples of the fundamental transmitted frequencies. Harmonic imaging utilizes these frequencies (generally only the second harmonic or twice the transmitted frequency) to produce an image. These images generally have improved axial resolution due to shorter wavelength, and better lateral resolution due to improved focusing with higher frequencies. They also have fewer artifacts, as the smaller amplitude of the harmonic waves decreases the detection of echoes from multiple scattering events; they also have less reverberation and side lobe artifacts, and increased contrast resolution as compared to standard sonography. This is particularly useful for obese and technically difficult patients. It also provides for a more accurate characterization of cystic lesions. The disadvantages of this technique are that the harmonic echoes are weaker, which can cause a noisier image. They also have less penetration than images obtained with conventional sonography. This technique is

available on most of the latest ultrasound machines (1,2,3).

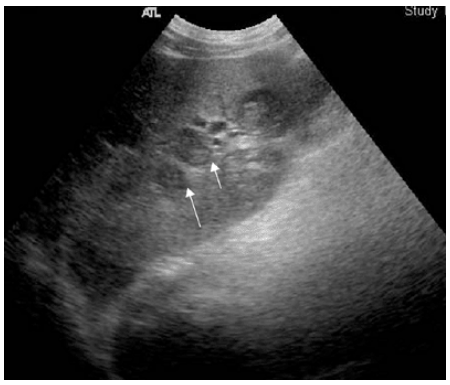




• **Figure 4.1** Simple hepatic cyst (*arrow*). Note the posterior acoustic enhancement.



• **Figure 4.2** Hemangioma. Transverse ultrasound image through the left lobe of the liver shows a hyperechoic (*arrow*) 2 cm hemangioma.



• **Figure 4.3** Hepatic metastasis. Saggital view through the left lobe of the liver shows four metastatic lesions (*arrows*) in a patient with metastatic colon cancer.

In conventional sonography, the liver is isonated at a single constant angle. In real-time compound sonography, the image is obtained by combining slices from multiple different angles. As the object is now imaged from multiple spatial orientation and the image is formed from the summation of the individual frames, this results in reduced image artifacts, better delineation of surface noise, and better image contrast to produce a better sonographic image. Limitations include blurring from motion, as the image takes longer to acquire. There is also less posterior acoustic shadowing, which is often a useful artifact when trying to characterize a lesion (4). It is possible to combine compound and harmonic imaging modalities; one study has suggested that this technique will produce images superior to either technique alone (5).

Ultrasound specific intravenous contrast agents are intravenous "microbubble" contrast agents that consist of an encapsulating shell material (albumin, phospholipid or polymer) surrounding air or flourocarbon gas. These particles are relatively stable in the bloodstream and highly reflective when isonated by ultrasound at typical frequencies used in medical imaging. In fact, there is linear reflection (at the same frequency as that transmitted by the transducer) and a non linear component of reflection that is useful for harmonic imaging (see the subsequent text). In general, the degree of increased echogenicity in a liver lesion will depend on the relative perfusion of the lesion compared to the liver (thus yielding information similar to a contrast-enhanced computed tomography (CT) scan or magnetic resonance imaging (MRI). These agents have not been approved by the U.S. Food and Drug Administration (FDA) for use in the United States, but they have been used in Europe for the past few years. Contrast-enhanced scans are thought to provide more information than color Doppler alone. Tissue harmonic imaging can detect nonlinear vibrations of microbubble contrast agents and is a fertile area for future research (6).

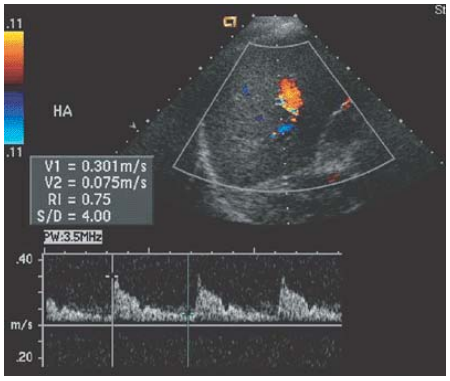
Given the relatively low sensitivity of ultrasound for the detection of liver lesions in patients with parenchymal liver disease, contrast agents offer the potential of bringing ultrasound to a comparable level with CT scan and MRI. A recent study utilizing contrast agents for

the detection of small hepatocellular carcinoma (HCC) reported sensitivity and specificity rates of 94% and 93%, respectively (7). In contrast, conventional ultrasonography has been reported to have a sensitivity of 74% to 88% for the detection of cirrhosis when liver biopsy and/or gross observation at laparoscopy were used as standards of reference (8,9,10).

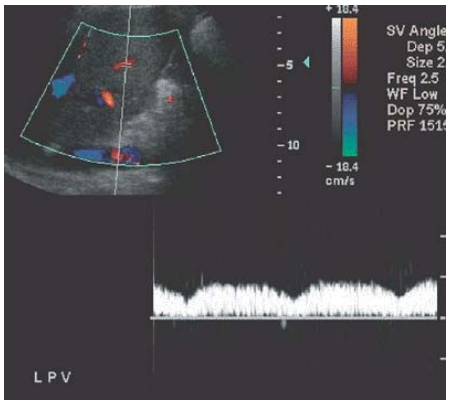
The reported sensitivity of conventional ultrasonography for the detection of metastatic lesions in the liver is relatively poor (53% to 77%) and is inferior to that of contrast-enhanced CT scan and MRI. Lesions are missed at ultrasound either because they are too small, with the sensitivity for lesions smaller than 1 cm being as low as 20%, or because the lesions are isoechoic with the background tissue and therefore very difficult to perceive. This also explains why it is difficult to detect lesions in cirrhotic and fatty liver. The parenchymal changes in these livers increase the reflectivity and they appear "brighter" on ultrasound and will therefore "hide" lesions, which are of the same echogenicity (11,12,13,14).

Color, spectral, and power Doppler imaging provide a noninvasive method of measuring flow in the hepatic blood vessels, and assessing vascularity within a lesion. Spectral Doppler provides a tracing of the Doppler wave from which we can calculate various indices, including peak systolic velocity and resistive indices (Fig. 4.4).

In conjunction with the color Doppler, it enables the operator to determine the direction of blood flow. Power Doppler imaging displays the integrated power of the color signal to depict the presence of blood flow. The color Doppler display reflects the mean Doppler frequency shift. Power Doppler is therefore a more sensitive method of demonstrating flow, but does not give any quantitative information about the velocity or the direction of blood flow (15,16). Nearly all ultrasound machines now have, as standard, color and spectral Doppler imaging. It is routine to document the presence or absence of flow, and the direction of flow in the hepatic veins and portal veins (Figs. 4.5 and 4.6). Interrogation of the hepatic artery is not routinely done except when specifically requested, or in a posttransplant patient.

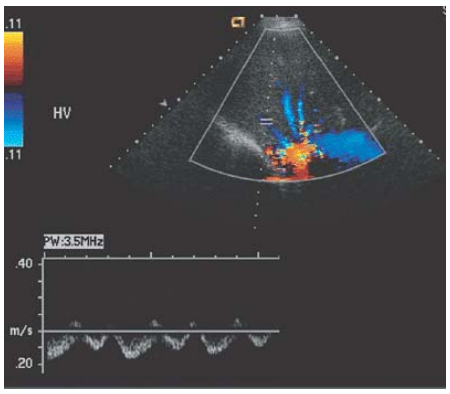


• **Figure 4.4** Hepatic artery. Spectral Doppler tracing of the right hepatic artery. Peak systolic velocity and minimum diastolic velocity values are used to calculate the resistive index.



• **Figure 4.5** Portal vein. Spectral Doppler tracing of the left portal vein shows a normal hepatopedal (above the baseline) flow into the liver. It is normally of low velocity with an undulating spectrum.

There are no absolute contraindications to ultrasonography. Relative contraindications include morbid obesity, in which it is often difficult for the sound waves to penetrate the patient to produce a diagnostic image. Patients with respiratory compromise may not be able to suspend respiration. As discussed in the preceding text, the ultrasound of the liver is limited if the patient has parenchymal disease such as steatosis or cirrhosis. In these patients this limitation should be documented on the radiology report, and if there is persistent suspicion the physician should be aware that a CT scan or an MRI must be performed for further evaluation.



• **Figure 4.6** Spectral doppler tracing of the right hepatic vein as it drains into the inferior vena cava. The normal triphasic spectral tracing reflects the pulsatility of the right atrium.

Patients will need to fast for 6 hours to reduce the amount of bowel gas so that the gallbladder and the biliary system can be assessed at the same time. The test, in general, will take approximately 30 minutes, and the patient will be asked to hold their breath repeatedly so the technologist can take images during suspended respiration.

Computed Tomography

Just as the introduction of CT scan impacted the detection of liver lesions, recent advances in CT scan have revolutionized the characterization of these lesions. Spiral and helical CT scan are terms that are used interchangeably. Helical CT scan is now performed almost universally, enabling the acquisition of thinner slices with improved spatial resolution more rapidly than conventional CT scan. The difference between helical and conventional CT scan is that spiral scanners are able to continuously rotate the x-ray CT scan tube, while the patient is moved through the scan plane. Therefore, instead of producing single slices, spiral scanners are able to acquire data over a volume of the patient (such as the craniocaudal extent of the liver), while the x-ray CT scan tube is continuously rotated.

Early helical scanners were able to rotate 360 degrees in roughly 1 second (1,000 ms gantry rotation) and used a single detector (single-slice CT scan) with a thickness of approximately 1 cm (1 cm spatial resolution). This single axial image was formed by the rotation of a single detector, coordinated with incremental CT scan table advancement and a pencil like CT scan source. The first generation CT scanners took 25 minutes to produce five views of the head. This progressed to a linear row of detectors with a fan beam, to a partial circle of detectors, and finally to the present, in which the patient is moved through the scanner and a helix of raw projection data is generated. Instead of a single row of detectors there are multiple rows of detectors. This is how the term multislice CT scan, originated. Multislice scanners are so named because they have more than one detector (between 2 and 64), and can therefore produce more than one slice per rotation. Currently, slices are as thin as 0.4 mm and can be acquired using gantry rotation times as low as 0.33 seconds (165 ms temporal resolution).

Most facilities now have helical CT scan available to them. At the time of writing, many facilities have 64 row scanners and there are plans to have up to 125 rows, followed eventually by volumetric scanning. These scanners allow images to be produced more quickly: For example, with a 16-row scanner we can scan the patient from head to toe in less than a minute. Therefore, we can scan through the

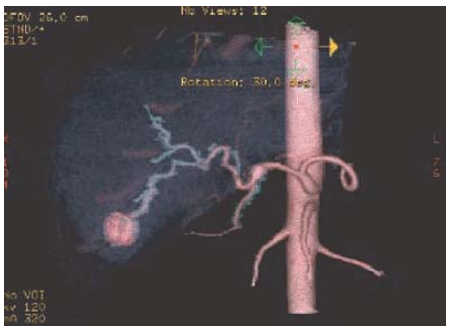
entire liver in less than 10 seconds, which allows scanning in multiple phases of enhancement, and also lessens the problems of respiratory artifact. These scanners can take thinner slices through the liver—with 0.625 mm slices; this allows almost isometric imaging (voxels of identical dimensions in all three planes) and exquisite three-dimensional (3D) images. These advantages also permit more advanced image processing, such as digital subtraction and CT scan perfusion (17,18).

Nearly all facilities now have helical scanners and can scan through the liver in multiple phases. 3D and CT angiography can be routinely performed. While 3D is not needed routinely for the assessment of a liver lesion, 3D imaging and CT angiography are very helpful in identifying the location of the lesion for the surgeon and for vascular mapping, and should be considered for patients who are candidates for surgery. (Figs. 4.7 and 4.8). CT angiography is now routinely used for the preoperative work up of living related donors for partial hepatectomy. This also allows liver volume estimation before surgery (Fig. 4.9).

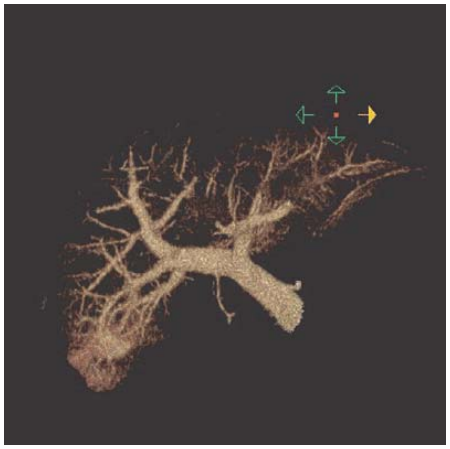
In summary, the development of multidetector CT scan has greatly advanced the image quality of hepatic imaging. CT scan offers the highest spatial and temporal resolution, allowing advanced applications and very high quality 3D imaging of both the parenchyma and hepatic vasculature. CT scan should be considered as the first option in older patients for whom radiation is not a concern. In particular, elderly patients who will have difficulty holding their breath will usually do better with CT scan than with MRI.

For a dedicated examination of the liver, patients need to fast for 6 hours. This has a twofold advantage; the gallbladder is distended which allows a better evaluation, and the patient has an empty stomach, which will reduce the risk of any vomiting post injection of intravenous contrast. There is usually no need to drink oral contrast for a dedicated hepatic CT scan, unless there is some reason to evaluate the bowel also. For an examination targeted to the biliary system, many centers are now advocating that the patient drinks a liter of water prior to the scan. This distends the bowel and helps to trace the course of the common bile duct

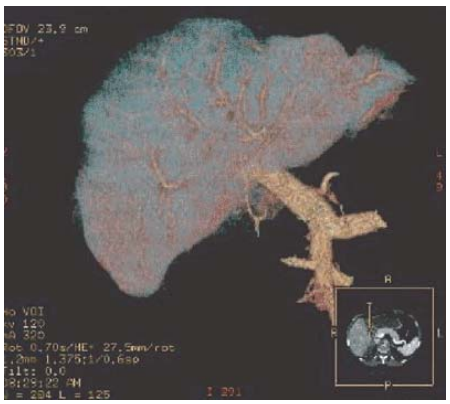
as it passes into the duodenum. For most examinations of the liver, patients will get an intravenous cannula inserted in their arm. The actual scanning time is approximately 5 minutes, but the patient should expect to be within the radiology department for about an hour. Most patients are observed for approximately 20 minutes for postcontrast reactions. If they have a history of allergy, they will need to be premedicated or an MRI scan considered.



• **Figure 4.7** 3D volume rendering of a liver with a small hepatocellular carcinoma in the right lobe (*arrow*). The computed tomography angiogram provides exquisite detail of the segmental branch of the right hepatic artery, which is supplying the tumor. This provides the surgeon with a clear road map to enable accurate preoperative planning.



• **Figure 4.8** 3D volume rendering of the same patient (fig. 4.7) now showing the relationship of the portal venous system to the tumor in the right hepatic lobe.



• **Figure 4.9** 3D volume rendering of the liver. The contour of the liver is traced on individual axial slices and then summed to give an accurate estimation of liver volume.

Relative contraindications include pregnancy, allergy to iodinated dye, renal failure, and patients with end stage cirrhosis who are at risk for hepatorenal syndrome.

Radiation

There has been much concern about radiation dose with CT imaging. If all medical x-ray imaging modalities are analyzed, CT scan accounts for 40% to 67% of the radiation dose, but only 5% of the number of tests ordered. The number of CT scan examinations being performed is increasing every year (19). Most of this concern is focused on the pediatric and younger populations, which are most sensitive to radiation. However, both the radiologist and the ordering physician should be aware of the radiation dose.

The unit for assessing the risk of cancer from a CT scan is the "effective dose" which is measured in millisieverts (mSv; 1 mSv = 1 mGy in the case of x-rays.) Effective dose allows comparison of the risk estimates associated with partial or whole-body radiation exposures. It

also incorporates the different radiation sensitivities of the various organs in the body. The actual effective dose from a CT scan can vary by a factor of 10 or more depending on the type of CT scanner, the protocol (how many phases, kVP, mAs etc.) and patient weight. Table 4.1 shows a list of radiology tests and their associated doses taken from a report of the European Commission (20). The reduction of the dose in general requires lowering mAs and kVp and increasing pitch. Therefore, there is always a trade-off between the image quality and the dose. Most radiology departments have dose reduction policies in place, particularly for the pediatric population (21).

Nephrotoxicity

The field of contrast usage has progressed from high osmolar contrast agents (osmolality five times higher than that of serum) to low osmolar contrast agents twice the osmolality of serum, and finally to iso-osmolar (290 mOsm/L) agents, which are almost isotonic with blood. They have been reported to have less nephrotoxic effects than the other contrast agents (22). Other approaches to reducing contrast induce nephropathy (CIN) have been the use of *N*-acetylcysteine. This has the advantage of being inexpensive, safe, and is widely used. Although, not all studies have shown a definite benefit, the use of *N*-acetylcysteine has become routine in many centers for patients with renal insufficiency. (23,24).

Computed tomography perfusion

The basis of CT scan perfusion is that it detects the difference between regional and global alteration in blood flow. It was initially developed in the brain to try to quantify blood flow for patients with a cerebrovascular accident. The ability to distinguish between arterial and portal venous phase on a global and regional basis imaging provide the basis for CT scan perfusion of the liver.

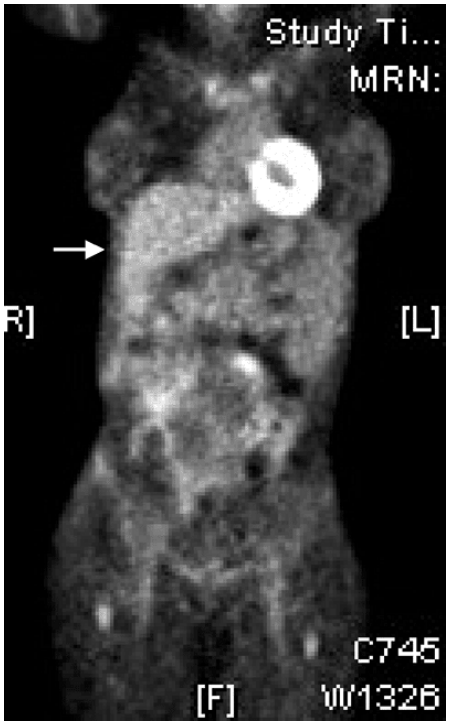
In order to perform this technique, the purchase of a separate software package is required. Perfusion measurement is the blood volume divided by time divided by tissue volume. For example, there is a relative increase in arterial versus portal venous flow in cirrhosis, offering potential for the early noninvasive detection of cirrhosis, but this remains to be proven. A similar rationale exists to offer the hope of earlier detection of HCC and metastatic disease. Dose considerations means that this technique is not yet feasible for these options (25). The technique of CT scan perfusion remains investigational, and is not available on a routine basis.

Positron Emission Tomography and Computed Tomography–Positron Emission Tomography

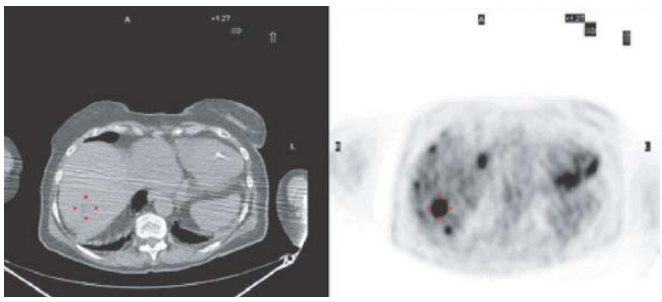
Positron emission tomography (PET) is a functional imaging modality. It utilizes positron emitters such as fluorine-18, oxygen-15, nitrogen-13 and carbon-11. However, all of the above except fluorine have very short half-lives, which means that an on site cyclotron would be required. Therefore, this discussion will be limited to the use of fluorine-18 which is widely available as 2-fluoro-deoxy-D-glucose (FDG). FDG has a half-life of 110 minutes and has high uptake in most cancers.

FDG-PET is based on the principle that there is increased glucose metabolism seen in malignant tissue relative to that of the surrounding normal tissue, and that this increase in metabolism is seen as a more intense uptake on FDG-PET images (Fig. 4.10). FDG competes with glucose for transport into a cell. Inside the cell, like glucose, FDG is phosphorylated by hexokinase into FDG-6-phosphate. In normal liver cells, there is a high concentration of glucose-6-phosphatase, which causes dephosphorylation and allows it to exit from the cells. Therefore, in the healthy patient there is rapid clearance of FDG from the liver. In malignant cells the up regulation of hexokinase causes increased glucose utilization (26).

These cells often have decreased glucose-6-phosphatase, and the FDG remains trapped within the cell (27,28,29). Increased FDG uptake is, however, nonspecific and may occur in any condition associated with increased tissue metabolism. This accounts for the uptake seen in acute or chronic inflammatory or infectious processes (30).



• **Figure 4.10** Normal positron emission tomography scan of the body demonstrating the normal uptake in the liver (*arrow*).



• **Figure 4.11** Positron emission tomography–computed tomography scan of a patient with multiple colorectal metastases. The dominant lesion in the right lobe of the liver is marked on both images (*arrows*).

PET–CT scan combines the cross-sectional anatomic information from CT scan with the functional information acquired by PET. They are both acquired during a single examination and fused. The ability to fuse information from both studies allows the accurate localization of increased FDG activity to specific anatomic locations. This is often difficult with PET alone (31).

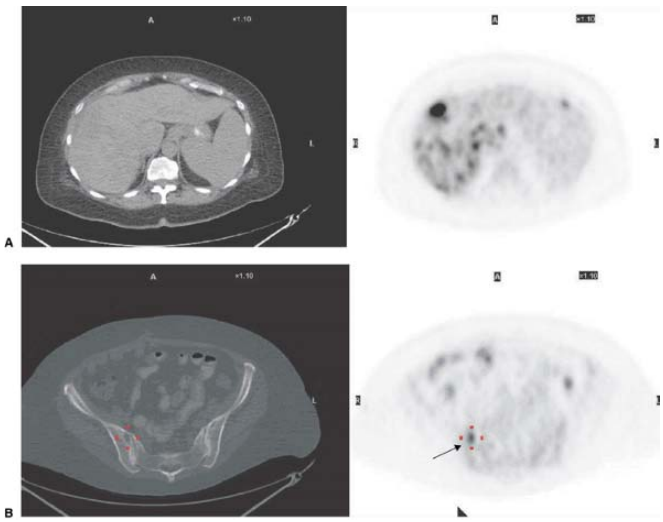
Hepatic metastatic disease

Studies utilizing PET have shown encouraging results when evaluating patients for metastatic liver disease. In studies of patients with a known solitary hepatic metastasis identified on conventional imaging, PET can detect other lesions. This is of particular importance in this patient population, as it may radically change patient management (32,33,34).

In a meta-analysis of the literature on colorectal liver metastases from January 1990 to December 2003, sensitivity estimates on a per-patient basis for helical CT scan, 1.5-T MRI, and FDG-PET were 64.7%, 75.8%, and 94.6%, respectively. On a per-lesion basis, sensitivity estimates for helical CT scan, 1.0-T MRI, 1.5-T MRI, and FDG-PET were 63.8%, 66.1%, 64.4%, and 75.9%, respectively. Therefore, FDG-PET had significantly higher sensitivity on a per-patient basis, compared with that of the other modalities, but not on a per-lesion basis (35). A meta-analysis looking at noninvasive imaging methods for the detection of hepatic metastases from colorectal, gastric, and esophageal cancers found that at equivalent specificity, FDG-PET is the most sensitive noninvasive imaging modality for the diagnosis of hepatic metastases from colorectal, gastric, and esophageal cancers (Fig. 4.11) (36).

False-negative FDG-PET can occur secondary to physiologic movements of the liver, infectious/inflammatory foci, underestimation of uptake, and recent completion of chemotherapy or radiotherapy. PET has demonstrated a high sensitivity for detection of disease when the lesions are larger than 1cm. As it does not reliably detect tumors less than 1 cm, it is suggested that PET-CT scan offers the patient the benefit of both imaging modalities (37,38). Most of the work on hepatic metastases has focused on patients with colorectal carcinoma.

One of the major advantages of PET is also the ability to survey the whole body for metastatic disease. Studies suggest that when PET is added to CT scan in preoperative planning for patients with hepatic metastases, additional sites of extrahepatic disease are identified in 11% to 23% of patients. This has had significant clinical impact in the management of these patients (39,40).



• **Figure 4.12 A:** Positron emission tomography–computed tomography (PET–CT) scan of a multifocal highly aggressive hepatocellular carcinoma. The PET scan shows the extent of the disease. **B:** In the same patient, images through the sacrum show a metastatic bone lesion in the right ileum. The PET–CT scan can help to detect unsuspected metastatic disease.

Hepatocellular carcinoma

HCC has a low uptake of FDG. Consequently, the detection rate of PET scans for HCC is approximately 50% (41,42). The uptake of FDG appears to correlate with the degree of differentiation of the HCC (Figs. 4.12A,B). Poorly differentiated tumors have greater uptake of FDG than well-differentiated tumors. This is thought to be due to the fact that in well-differentiated HCC, there is a high intracellular concentration of glucose-6-phosphatase so that well-differentiated HCC and normal hepatocytes metabolize glucose-6-phosphate and FDG at the same rate (43). Also, glucose transporters do not seem to be overexpressed in HCC as often as in other malignancies (44).

11-C acetate is a newer PET tracer with a half-life of 20 minutes. Therefore, an on site cyclotron would be required to utilize this tracer. In contrast to FDG, it

is taken up in well-differentiated HCCs, but it cannot detect poorly differentiated HCCs (45). Recent studies have suggested that the delayed imaging of the liver at 2 to 3 hours may offer improved results for the detection of HCC. This is thought to be due to a gradual increase in uptake in FDG by the tumor, and decreased uptake by the hepatocytes.

In summary, FDG should not be used for the routine detection of HCC. FDG uptake depends on the histological grading of the tumor and is highest in the poorly differentiated tumors, whereas 11-C acetate uptake is better for the well-differentiated tumors. Delayed scans post injection may offer a better sensitivity.

Cholangiocarcinoma

The largest study (31 patients) looking at the use of PET for the detection of cholangiocarcinoma showed a sensitivity of 61% and a specificity of 80%. It showed a better sensitivity for nodular than infiltrating lesions. Care must be exercised in patients with biliary stents as they can cause a false-positive reading. Acute inflammation in patients with sclerosing cholangitis can also cause a false-positive reading. The authors suggest that PET is accurate in predicting the presence of nodular cholangiocarcinoma (mass >1 cm) but is not helpful for the infiltrating type (46). Other studies have shown decreased sensitivity for the detection of mucinous tumors (47,48). Hilar and extrahepatic cholangiocarcinoma are less intense on FDG-PET than peripheral cholangiocarcinoma (49,50). The use of standardized uptake values for evaluating the uptake of FDG shows promising results in trying to distinguish between extra hepatic bile ducts malignant and benign strictures (51).

In summary, it appears that if cholangiocarcinoma has sufficient tumor volume, it will take up FDG-PET. It is not sensitive for mucin containing tumors and will have false-positive results for those patients with inflammatory conditions such as sclerosing cholangitis.

Radiation dose

One study has reported the effective dose per PET–CT scans examination at or about 25 mSv. Please see Table 4.1 for comparison to other examinations (52,53,54).

Patient information

Although each center will have its own specific procedures, the following are general guidelines. The PET scan and the CT scan will be performed during a single visit. Patients should fast for at least 4 to 6 hours prior to their scan. They will receive an intravenous injection of 18FDG, following which there is usually a wait of about an hour for the FDG to distribute throughout the body. The patient is then taken to the scanner. The CT portion of the scan is usually completed first. This will take only approximately 5 minutes. Depending on the part of the body being imaged, patients may receive another injection at this stage of radiographic contrast. Intravenous radiographic contrast is not used routinely at present. On the same imaging table, the PET portion of the scan is then performed. This may take up to 45 minutes.

Table 4.1. Typical Effective Doses of Ionizing Radiation from Common Imaging Procedures (20)

Class	Typical effective dose (mSv)	Examples
0	0	US, MRI
I	<1	Chest x-ray
II	1–5	IVU, lumbar spine x-ray, CT head and neck
III	5–10	CT chest and abdomen, cardiac nuclear medicine studies
IV	>10	PET studies

US, ultrasonography; MRI, magnetic resonance imaging; IVU, intravenous urogram; CT, computed tomography; PET, positron emission tomography.

If the blood sugar is elevated over 200 mg/dL, the scan will be postponed. All diabetic patients must have their blood sugar levels regulated. Insulin should not be injected near the time of the scan, as it will cause increased uptake in the local skeletal musculature. It is recommended that FDG-PET be performed in the morning after an overnight fast. The patient should not exercise for 24 hours prior to the scan. The patient's bladder should be emptied prior to the scan. The patient should be asked about recent surgery, active inflammation, or infection.

Magnetic Resonance Imaging

State of the art MRI of the liver is now routinely performed with a torso phased array coil. This is a surface coil placed on the patient which provides a better image by allowing a greater signal-to-noise ratio. Stronger magnets and improved software now allow imaging of the entire liver in one breathhold. Multiple sequences are performed in a routine liver MRI. These sequences should include T1, T2, in-and out-of-phase images, and pre– and post–dynamic contrast images. The pre- and post contrast sequence can be performed either with standard 2 dimensional axial sequences, or a 3D volume acquisition.

The advantages of MRI are the lack of radiation and the use of non-nephrotoxic contrast material. It offers higher contrast resolution but decreased spatial resolution when compared to CT scan. Nearly all liver

examinations require administration of contrast. However for an MRCP, no contrast is given. Therefore, if the sole goal of the test is to evaluate the bile ducts for calculus disease, contrast is not required. However, if a simultaneous evalution of the pancreas or liver parenchyma is desired, or if there is any concern about malignancy, the study will require dynamic administration of intravenous contrast.

MRI is the test of choice for the noninvasive quantification of liver iron. The presence of iron causes a decrease in the MRI signal intensity causing the liver to appear dark. This is caused by a decrease in the T2 relaxation rate of protons. Other limitations are that the amount of fibrosis and inflammation in the liver can affect liver iron measurements (55).

Contrast agents

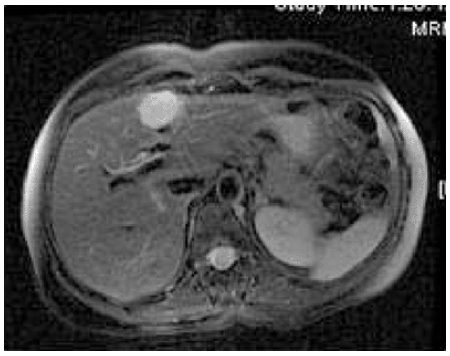
The vast majority of liver MRI is performed with gadolinium chelate contrast agents, which have characteristics similar to the contrast agents used for CT scan examinations. Newer contrast agents are liver targeted. The three different types of contrast agents are nonspecific gadolinium chelates, reticuloendothelial system specific agents, and hepatocellular-specific agents.

Nonspecific gadolinium chelates are the contrast agents routinely used in liver MRI examination. They are nonspecific extracellular

gadolinium chelates. It is a paramagnetic substance, which causes increased signal in tissue on the post injection T1-weighted images. The agent initially distributes in the intravascular compartment, and then diffuses into the extracellular space. This enables the characterization of the tumor vascularity. It is safe, relatively low cost, and has good patient tolerance.

Iron oxide particles are taken up into the reticuloendothelial system (RES). In the liver, they are taken up by the Kupffer cells. Reticuloendothelial system specific agents (superparamagentic iron oxide particles) are administered as a slow infusion over 30 minutes. The most common side effects are hypotension and lumbar pain. Most focal liver lesions lack Kupffer cells. The normal liver will appear dark on T2-weighted images due to the normal uptake of the particles—thereby increasing sensitivity for the detection of focal liver lesions (Fig. 4.13). The lesions cannot be characterized as benign or malignant (56). Newer agents with ultra small iron oxide particles also offer dynamic imaging as they are taken up more quickly into the Kupffer cells. They offer the possibility of characterization of lesions, as lesions containing Kupffer cells will take up the contrast agent (57).

Hepatocellular-specific agents, including newer agents such as mangafodipir trisodium, are administered by slow infusion, accumulate in hepatocytes, and are excreted by the biliary system. This causes an enhancement of the liver parenchyma, but not of masses of nonhepatocytic origin. This may lead to greater detection of lesions. This has been shown to be most useful when assessing patients with liver metastases to determine if they are viable surgical candidates. It does not, however, help to characterize the lesion as benign or malignant. As HCCs contain hepatocytes; these agents are not useful for the detection of HCC. It has also been postulated for use to evaluate the biliary system, such as for the detection of biliary leaks (58,59,60,61).



• **Figure 4.13** T2-weighted axial image through the liver shows a left lobe hemangioma in a patient who has received ferridex. Note the low signal intensity of the liver.

Combinations of perfusion and hepatocellular agents combine the properties of a conventional extracellular contrast agent with those of a liver specific contrast agent: Examples of these agents are gadobenate dimeglumine (Multihance Bracco Diagnostics, Princeton, New Jersey) and gadoxetic acid disodium (Eovist Schering, Berlin, Germany). They are initially distributed in the vascular interstitial compartment like the normal extracellular gadolinium chelate contrast agents, and then a percentage is taken up into the hepatocytes in a delayed fashion. Therefore, they can offer both dynamic characterization and the opportunity for increased liver lesion detection (62,63,64).

Summary

Characterization of liver lesions with standard extracellular contrast agents remains the standard of care in the United States. In Europe, however, the use of liver specific contrast agents now offer the possibility of a one-stop test to evaluate focal liver lesions.

A dedicated liver MRI examination will require at least 30 minutes in the magnet. This involves multiple breath holds. It is a much longer examination than a CT scan of the liver. This is often stressful for elderly patients or patients with a large amount of ascites

who are unable to successfully suspend respiration for 20 to 30 seconds in a repetitive fashion for this time period. In these patients a CT examination should be considered. However for the younger patient where radiation is a concern, or where there are renal problems, MRI is the test of choice. Nearly all patients will require an intravenous injection. This is approximately 20 mL, plus a saline flush of a gadolinium based contrast material. The bore of the magnet is relatively small but most patients, unless claustrophobic, tolerate the examination. The presence of a pacemaker or ferromagnetic materials in a patient is a contraindication to performing an MRI.

Selected Clinical Problems

Nonalcoholic Fatty Liver Disease

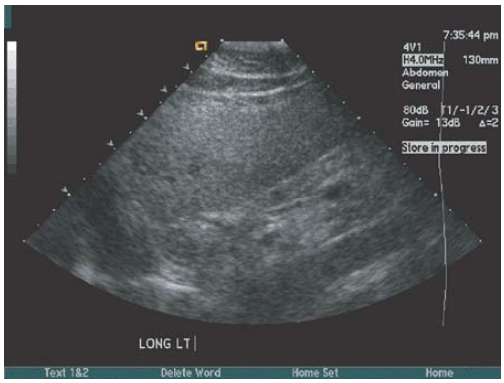
Exceeding by a wide margin the prevalence of any other liver disease, nonalcoholic fatty liver disease (NAFLD) occurs in about 20% of people in the United States. In recent years, increasing evidence has accumulated supporting the potential for NAFLD to cause progressive hepatic fibrosis with all its attendant complications (65), including HCC. A distinction has been made between steatosis alone, which appears to have a relatively benign prognosis and is the more prevalent of the two variants of NAFLD and nonalcoholic steatohepatitis (NASH), which is the variant that carries greater potential for progressive fibrosis (66,67). Thus, distinguishing between steatosis and steatohepatitis is a key component of the evaluation of patients with NAFLD.

One of the leading dilemmas faced by clinicians in evaluating patients with suspected NAFLD is the role of liver biopsy. A consensus that liver biopsy is required for all patients with this disorder would lead to a marked increase in the number of biopsies and would possibly be unrealistic in terms of the available resources. Imaging has the potential to play a critical role in the management of such patients. Among the leading questions are the following: (i) What is the sensitivity and specificity of imaging in the diagnosis of NAFLD in patients with elevated liver enzymes who have no evidence of other liver diseases? (ii) Can imaging be used to distinguish steatosis from steatohepatitis? (iii) To the extent that imaging has a role, which imaging modality is most useful? (iv) Can imaging be used to follow patient response to therapeutic interventions? These questions will be considered in the discussion that follows.

Detection of fatty liver by imaging

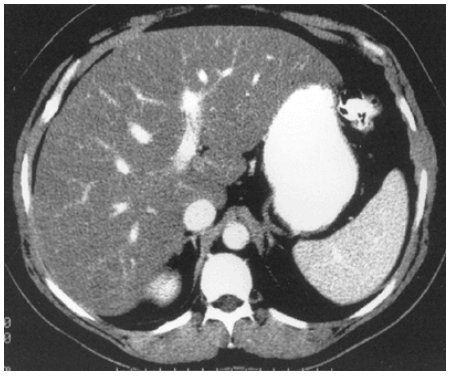
On ultrasound, fatty liver can be recognized by several characteristics, including increased parenchymal echogenicity, or a bright echo pattern which accentuates a normally inconspicuous difference between the liver and the renal cortex or the spleen (Fig. 4.14) (68); increased attenuation of the ultrasound beam, causing posterior darkness and loss of definition of the diaphragm; and loss of intrahepatic architectural detail producing loss of definition of the portal veins (68). The loss of portal vein definition occurs because the fat normally surrounding the portal veins, which usually offsets the veins from the parenchyma, now has the same echogenicity as the liver. The increased echogenicity caused by fibrosis has led to the term “fibrofatty pattern” (69). The nonspecificity of this pattern in distinguishing fibrosis from fat is one of the limiting features in the use of ultrasound for fatty liver disease. Furthermore, increased hepatic echogenicity is not specific for hepatic steatosis and may be seen in other diffuse liver diseases. Attempts to grade the severity of steatosis using

ultrasound criteria are limited by the generally broad categories described, and by the operator dependency of the technique and differences due to ultrasound settings used (69,70).



• **Figure 4.14** Fatty liver. Ultrasound image shows an echogenic, attenuating liver.

The accuracy of CT scan in detecting fatty liver is greatest with noncontrast studies. Contrast-enhanced CT scan is less accurate for the diagnosis of hepatic fat deposition because of the varying densities of the liver and spleen on these images, and the interference of the contrast with fat detection (71). In contrast to sound waves, fatty liver results in the *decreased* attenuation of x-rays, resulting in a darker appearance than normal and, in a reversal of the usual pattern, a liver which is darker than spleen. Fatty liver is diagnosed on CT scan if the liver attenuation is less than 40 Hounsfield units or a relative attenuation of 10 Hounsfield units less than the spleen (Fig. 4.15). The spleen is used as the reference organ as it does not accumulate fat (72,73).



• **Figure 4.15** Fatty liver. Computed tomographic scan shows diffuse low attenuation to the liver consistent with fatty infiltration.

Other diffuse liver diseases, including acute hepatitis, may also cause decreased hepatic attenuation. There is a relationship between the degree of hepatic steatosis and CT scan density but, as for sonography, a reliable distinction of steatosis from steatohepatitis cannot be made with CT scan, nor can the degree of fibrosis be reliably assessed (68,74,75). Although the radiation exposure with CT scan places practical limits on the widespread use of serial assessments for hepatic steatosis with this technique, some studies have used serial CT scan for assessing the response of hepatic steatosis to therapy (76).

MRI quantifies the fat content of the liver by chemical shift imaging, which takes advantage of the difference in resonant frequencies between protons in a fat and water environment. A recent study has described the successful quantitation of hepatic fat morphology and the severity of steatosis with MRI (77). Chemical shift imaging is not confounded by the presence of hepatic fibrosis (77). The liver is imaged on a T1-weighted gradient dual echo sequence using both "in-phase" and "out-of-phase" images. In steatosis there is a decrease in the MR signal intensity on the "out-of-phase" images. Comparison is performed with the spleen, which does not usually accumulate fat. However, the spleen can accumulate iron, which will cause the signal intensity to decrease. In this situation, the skeletal muscle or the kidney may provide a more accurate comparison for the assessment of changes in hepatic signal intensity.

Magnetic resonance spectroscopy (MRS) allows the quantitation of hepatic fat that can be used for serial estimations without the radiation exposure of CT scan. Studies in patients with both alcoholic and nonalcoholic fatty liver disease have reported accurate measurements of hepatic fat deposition with this technique (78,79).

Performance characteristics of imaging modalities

In a recent analysis of the literature, the sensitivity of ultrasound for recognizing fat in the liver ranged from 60% to 89%, and the specificity was 84% to 95%. The positive predictive value for an ultrasound suggesting fatty liver was 96%, while an ultrasound not demonstrating fatty liver was associated with a 19% chance of fatty liver being present (68). The ultrasound criteria for the determination of fatty liver has varied in these published studies with some requiring only increased hepatic echogenicity (without comparison with the echogenicity of other organs) for diagnosis. More strict criteria for the ultrasound diagnosis of steatosis and more severe degrees of steatosis result in improved performance characteristics (70,80). A study of 187 morbidly obese patients compared ultrasonography with liver histopathology from liver biopsies done at the time of bariatric surgery. In this patient population, the sensitivity, specificity, and positive predictive value for ultrasound in detecting hepatic steatosis was 49%, 75%, and 95%, respectively (81).

On CT scan there is a good inverse correlation between the degree of steatosis and CT scan density scores (68). In a series of 25 consecutive patients with biopsy-proven steatosis undergoing sonography, CT scan and MRI, the presence of greater than 33% fat on liver biopsy was optimal for detecting steatosis on radiological imaging (82). Ultrasound and CT scan were 100% and 93% sensitive in detecting greater than 33% fat, with positive predictive values of 62% and 76% respectively. The degree of intraobserver and interobserver

agreement was best for MRI, but none of the modalities were able to distinguish steatosis from steatohepatitis, leading to the authors' conclusion that liver biopsy remains the "gold standard."

In a direct comparison of MRI with ultrasound, MRI was more sensitive than ultrasound in detecting relatively small degrees of steatosis, and correlated better with microscopic fat content. MRI correlated better with macrovesicular steatosis than mixed macrovesicular and microvesicular steatosis. Unlike ultrasound, with which several patients with severe fibrosis were misinterpreted to have severe steatosis despite low levels of hepatic fat content on biopsy, MRI was not influenced by the presence of fibrosis (77).

A combination of proton ^{1}H MRS and total body MRI was studied in 11 subjects with biopsy-proven hepatic steatosis and in 23 healthy volunteers (83). Intrahepatocellular lipid signals were detectable in all subjects but were significantly greater in hepatic steatosis than in healthy volunteers, and were greater in overweight than in lean subjects. In addition to the relationship between hepatic fat content and total

body fat as a percentage of body weight, the strongest correlation was between central obesity and hepatic fat content. The authors proposed that a combination of their technique with ^{31}P MRS, which reflects cell membrane turnover and fibrosis, might in the future help resolve the current limitations of noninvasive imaging modalities in distinguishing steatosis from steatohepatitis with fibrosis (83).

Role of imaging in practice

Hepatic imaging has a limited but useful role in the evaluation of patients with unexplained liver enzyme abnormalities. If the history and serologic tests are negative for evidence of alcohol, hepatotoxic drugs, toxins or viral, genetic, and autoimmune liver diseases, an ultrasound revealing an echogenic liver or other characteristics of fatty liver disease strongly implies NAFLD or NASH—particularly if there is no clinical, physical, or laboratory evidence of advanced fibrosis, which may confound the interpretation of ultrasound for steatosis. At that point, the decision as to how to proceed depends upon the therapeutic options perceived to be available. If the liver enzyme abnormalities are mild and without evidence of hepatic functional compromise, the patient appears well, and there are noninvasive avenues available for intervention (e.g., overweight status, modest alcohol consumption, or hyperlipidemia) it is reasonable to address these issues for a period of time and observe the effect on the liver tests. If there are worrisome clinical or laboratory features, or if noninvasive approaches have been exhausted, a liver biopsy to distinguish steatosis from steatohepatitis is appropriate.

Since all imaging modalities are limited by the inability to distinguish steatosis from steatohepatitis, the threshold for proceeding to biopsy will vary among clinicians and their therapeutic approach to patients with fatty liver disease, especially steatohepatitis. For example, the frequency of liver biopsy to assess for NASH is likely to depend substantially upon the evolving evidence that specific treatments for steatohepatitis, for example, insulin-sensitizing drugs, are effective, and whether in the next several years such agents will continue to be investigational, or are officially approved for such use. With the increased understanding of the pathophysiology of fatty liver disease, it is highly likely that additional new treatments will be developed, leading to a greater reliance upon liver biopsy when there are clinical and/or radiologic findings suggesting hepatic steatosis. On the other hand, it is hoped that there will be progress in combining imaging with serum markers, such as fibrosis assays, that will help make the distinction between steatosis and steatohepatitis, a trend that would further entrench imaging in a central role in the evaluation of these patients.

If a patient with unexplained liver test abnormalities has no evidence of steatosis on ultrasound, there is still a potential role for liver biopsy, since the positive predictive value of steatosis on sonography is greater than the negative predictive value. However, negative imaging in the setting of unexplained liver disease increases the suspicion for other liver diseases, and may therefore provide an additional impetus for liver biopsy. With further research and experience MRI, possibly in association with MRS as suggested in the preceding text, may play an increasing role given its capacity to detect smaller amounts of hepatic fat than ultrasound.

Impact of fatty liver on accuracy of hepatic imaging

It is now widely accepted that patients with cirrhosis from a variety of causes, including hepatitis B, hepatitis C, hemochromatosis, alcoholic liver disease, nonalcoholic fatty liver disease, α_1-antitrypsin deficiency, primary biliary cirrhosis, and others, should undergo noninvasive hepatic imaging periodically to screen for evidence of early HCC. Although data from prospective controlled trials are limited, there is near unanimity among hepatologists and surgeons that early detection does favorably impact upon prognosis. Indeed, the presence of HCC increases the Model for End-Stage Liver Disease (MELD) score substantially, propelling the patient forward on the transplant waiting list.

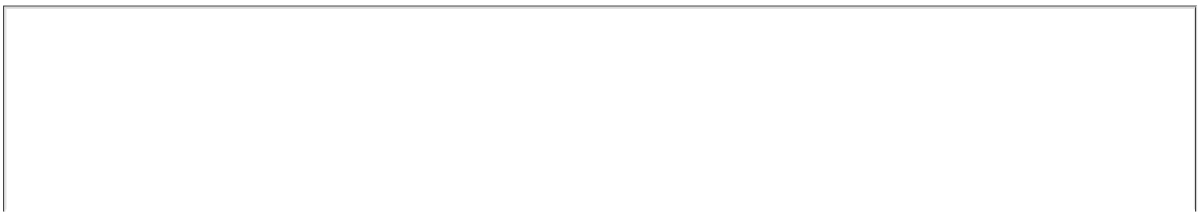
From a cost-effective viewpoint, ultrasound is generally used as the most common hepatic imaging modality for HCC screening, and is combined with periodic determination of serum α-fetoprotein level. However, as is discussed in a separate section, ultrasound is not as sensitive as MRI with gadolinium or CT scan with intravenous contrast, raising the question of when one of the other modalities should be used. The situation in which ultrasound suffers the greatest decrement in relative sensitivity is in patients with fatty liver or in obese patients. Accordingly, some clinicians resort to MRI or CT scan for HCC screening in such patients. It is a matter of clinical judgement how frequently one of the alternative techniques should be obtained relative to ultrasound. When cost is not an issue, an argument can be made for exclusive reliance upon CT scan and/or MRI, bypassing ultrasound completely, particularly when the radiologist explicitly points out in an ultrasound report that sonographic visualization of the liver was limited. Another approach, when the ultrasound images are satisfactory but suboptimal, is to alternate CT scan or MRI with sonography such that one or the other is done every 6 months on a rotating basis. At present, however, periodic ultrasound remains an accepted standard. When the other imaging modalities are incorporated into a surveillance program, an

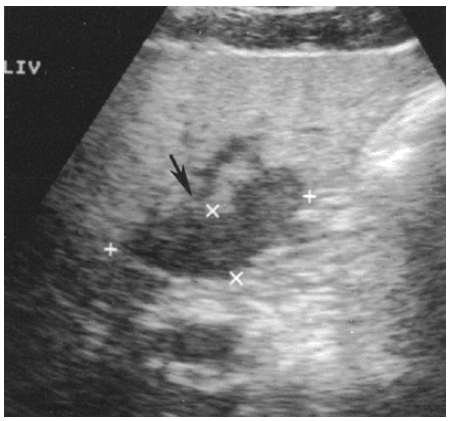
MRI has the advantage over CT of avoiding repeating doses of ionizing radiation over what may prove to be a long period of time.

Focal fat sparing and focal fat infiltration

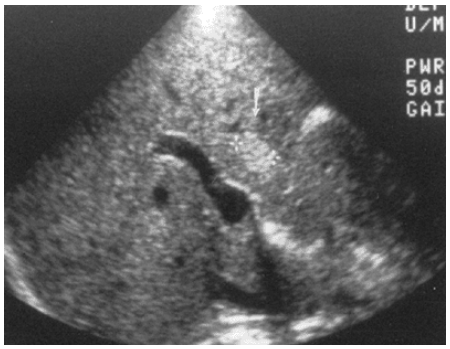
Although hepatic fat deposition often occurs fairly uniformly throughout the liver, focal fat deposition or focal sparing of an otherwise fatty liver are not uncommon, and result from aberrant blood supply. These conditions may cause diagnostic confusion by raising the possibility of a focal neoplastic lesion. Focal fat sparing is thought to occur because of gastric venous or other aberrant blood flow into the affected area, instead of blood flow from the portal system, with its lipid-rich blood supply. Common areas affected by focal fat sparing include the medial segment of the left lobe of the liver, adjacent to the gallbladder fossa, and the porta hepatis. These areas appear hypoechoic on ultrasound and hyperdense on CT scan in comparison with the rest of the fat infiltrated liver (Fig. 4.16).

Focal fatty infiltration occurs as a result of excess triglyceride deposition. These areas appear hyperechoic on ultrasound and hypodense on CT scan (Fig. 4.17). Common locations for focal hepatic steatosis include liver tissue adjacent to the falciform ligament, gallbladder or liver capsule, and the medial segment of the left lobe of the liver. In some instances, an area of increased fat deposition in an underlying fatty liver may also give the appearance of a focal lesion. Focal fat deposition is sometimes multifocal, mimicking metastatic disease. Chemical shift MRI using T1-weighted gradient echo sequences is useful in this situation (84).





• **Figure 4.16** Focal fatty sparing. Ultrasound image shows hypoechoic apparent mass (*arrow*) in a patient with hyperechoic fatty liver. Subsequent magnetic resonance imaging confirmed the presence of focal sparing from fatty liver.



• **Figure 4.17** Focal fatty liver. Ultrasound scan shows a focal echogenic mass (*arrow*) confirmed with magnetic resonance imaging to represent focal fatty liver.

Areas with either focal fatty sparing or deposition occur in typical locations, have sharply demarcated boundaries, angular margins, and do not exhibit a mass effect or vascular involvement, allowing differentiation from neoplastic and other lesions.

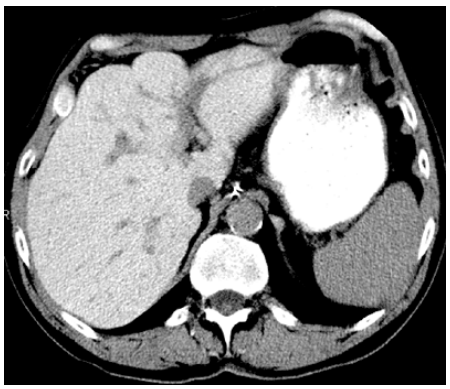
Imaging for Hepatic Iron Overload

Determination of hepatic iron status is important for the diagnosis of iron overload, to help decision making on whether treatment is needed, and to help determine response to therapy. The gold standard for hepatic iron determination is a liver biopsy, which can quantify the amount of iron in the liver. However, this test has limitations, including its invasive nature with the risk of significant complications. Variability in measured hepatic iron from small liver biopsy tissue samples, and in patients with cirrhosis, may also limit the usefulness of information gathered from this test. Therefore, noninvasive methods to assess hepatic iron are needed. CT scan has been investigated, but the most promising imaging techniques are MRI and magnetic susceptibility measurements.

Computed tomography

On CT scan, hepatic iron deposition results in an increase in liver density, usually measuring between 70 and 130 Hounsfield units (85). This appearance of the liver is a consequence of increased x-ray density due to greater density of iron compared with normal liver tissue (Fig. 4.18). However, a normal attenuation does not exclude hepatic iron overload. CT scan will

generally not demonstrate an attenuation difference until there is five times more than the normal iron contrast.



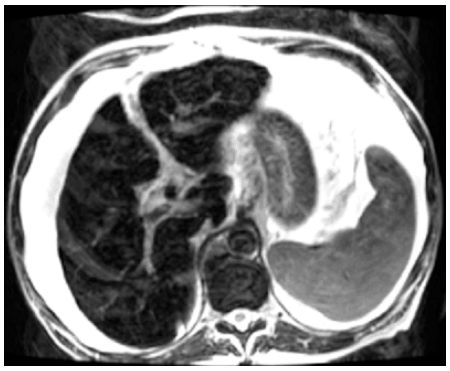
• **Figure 4.18** Hemochromatosis. Nonenhanced computed tomography demonstrates diffuse hyperattenuation of the liver parenchyma secondary to increased iron stores within the liver.

Guyader et al. compared 46 patients with hemochromatosis with 32 patients with chronic liver disease, and 22 controls using hepatic iron quantification as the reference method. Despite a high specificity (96%), the measurement of hepatic density on CT scan had a low sensitivity (63%) (86). In this study, CT scan did not detect iron overload in 14 of 18 patients with low and moderate hepatic iron overload (liver iron concentration <150 μmol/g dry liver weight). Steatosis also affected liver attenuation in this study. Others have also shown that steatosis and hepatic fibrosis affect the sensitivity of iron detection by CT scan (87,88). Moreover, an increase in hepatic density on CT scan is a nonspecific finding and is also seen in other conditions, including gold or thorotrast deposition (89,90), type IV glycogen storage disease (91), Wilson disease (92), and with certain medications including amiodarone (93).

Magnetic resonance imaging

MRI has been shown to be a more sensitive test for the detection of hepatic iron overload than CT scan (86,94,95). MRI takes advantage of the paramagnetic effect of iron to indirectly assess iron accumulation. The paramagnetic effect of iron has a T2-shortening effect, which causes a decrease in signal intensity on T1- or T2-weighted images. As a result, the liver appears darker than the spleen and other parenchymal organs on T2-weighted images (Fig. 4.19).

In efforts to optimize the accuracy of the assessment of hepatic iron, investigators have studied MRI performance specifications including the use of T2-weighted images, MRI magnet strength, the standard with which to compare hepatic signal intensity, and gradient recalled echo (GRE) versus spin-echo (SE) sequences. Higher strength magnets (1.5 T) provide more accurate hepatic iron assessments, particularly for lower levels of iron overload (94,96,97,98). Standard MRI utilizes SE sequences which are sensitive but not specific for distinguishing mild from severe iron deposition (99). GRE sequences are more sensitive to the inhomogenities induced by the paramagnetic effects of iron (100), and therefore provide more accurate estimations, particularly for low levels of hepatic iron.

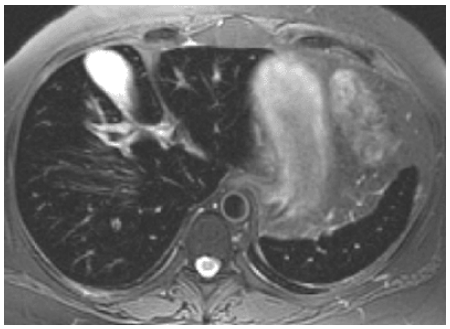


• **Figure 4.19** Hemochromatosis. T2-weighted magnetic resonance image demonstrating decreased signal intensity of the liver in comparison to the spleen secondary to increased tissue iron content.

Hepatic signal intensity is compared with other tissue standards to calculate signal intensity ratios used to make hepatic iron determinations. The normal liver signal intensity lies between that of the higher signal spleen and the lower signal muscle. When there is iron overload, the liver signal intensity becomes as low or lower than that of muscle. Conflicting results have reported optimal signal intensity ratios comparing the liver with paraspinous muscle (95,101,102), fat (100), the spleen (86), and background noise (97). Studies with 1.5-T MRI using liver to muscle signal intensity ratios have reported sensitivity and specificity rates of 89% to 100% and 74% to 80%, respectively, for hepatic iron detection (103,104).

MRI has been reported to be capable of differentiating hepatic parenchymal iron overload seen with primary or hereditary hemochromatosis from reticuloendothelial iron overload seen with secondary hemochromatosis (105). Parenchymal iron deposition results in low signal intensity on T2-weighted SE or GRE images. In patients with hemochromatosis with pancreatic and hepatic parenchymal iron deposition, there was a decrease in signal intensity in the

pancreas, compared with the skeletal muscle standard. In contrast, patients with secondary, reticuloendothelial system iron overload, there is a tendency toward decreased splenic, but not hepatic or pancreatic, signal intensity (Fig. 4.20). Rocchi et al. found that genetic hemochromatosis was associated with a dark appearing liver on T2 MRI images, while in patients with secondary or transfusional iron overload, the spleen and bone marrow also appear black, reflecting iron deposition in these organs as well (94). In patients with advanced iron overload, however, it is usually difficult to determine whether the etiology is primary or secondary iron overload.



• **Figure 4.20** Posttransfusion hemosiderosis. T2-weighted fast spin-echo magnetic resonance image demonstrates diffusely decreased

signal intensity of the liver and spleen secondary to increased iron content of tissue.

Olynyk et al. reported the use of MRI to assess the degree of fibrosis in patients with hemochromatosis (106). They initially evaluated the use of hepatic iron concentration (HIC) to predict the presence of low grade versus high grade fibrosis, and reported a sensitivity of 100% and a specificity of 67%. Using the product of the HIC and the patient's age, which allowed an estimation of the duration of hepatic iron exposure, resulted in higher specificity (86%) rates. Further studies are necessary to confirm this method.

MRI has several advantages over liver biopsy, including the noninvasive nature of the test, which is especially important in patients with bleeding disorders or decompensated liver disease. In these patients a liver biopsy may be contraindicated, while MRI has the ability to evaluate other organs that may be affected by iron deposition, include the pancreas and the heart (107), as well as capacity to perform serial evaluations and to simultaneously screen for HCC.

MRI can be used as a screening test to exclude iron overload in patients with low or moderate suspicion. Performance of an MRI is difficult in patients with claustrophobia, and the test is limited by the inability to provide information on hepatic histology, including the degree of hepatic fibrosis.

Quantification of iron and assessment of response to therapy

In addition to the detection of iron overload in the liver, the estimation of the degree of iron accumulation has practical implications. Quantification of hepatic iron content provides information about the degree of iron accumulation and can be used to follow response to therapy with either phlebotomy or chelating agents. Serial liver biopsies are not practical because of safety concerns and the risk of complications, but serial MRI images can be helpful.

A number of studies have reported accurate noninvasive quantification of hepatic iron using MRI (97,108). The logarithm of signal intensity and the transverse relaxation rate of images acquired using both gradient echo and SE techniques have a linear correlation with liver biopsies (97). There are limitations of hepatic iron quantification, including a lack of standardization between MRI machines and variations according to specific MRI technique. The presence of hepatic fibrosis and steatosis may affect the accuracy of MRI assessments of hepatic iron. While some investigators have reported that the presence of cirrhosis or steatosis do not significantly affect iron quanitification by MRI (100,103), others have come to different conclusions (109). The test may also be less accurate at extremes of hepatic iron deposition. Gandon et al. reported accurate hepatic iron estimations in the range of 80 to 300 μmol/g. Quantification of iron levels greater than 300 μmol/g was not possible because of very low liver signal intensities (100).

Serial MRI may also be useful in assessing the response to therapy for iron overload. A number of investigators have shown an increase in hepatic signal intensity in response to both phlebotomy (110) and chelation therapy (94,111). Jensen studied 11 nonthalassemic patients with transfusional iron overload who underwent chelation therapy with desferrioxamine (112). Hepatic iron quantification (39 to 626 μmol iron/g) was done using MRI 1.5 T, SE images, and signal intensity ratios between liver tissue and skeletal muscle. MRI hepatic iron quantification appeared to be a more accurate estimation of response to chelation therapy than monitoring serum ferritin levels.

Magnetic susceptibility measurement

A recently described technique is superconducting quantum interference device (SQUID) biomagnetic liver suseptometry testing which takes advantages of the paramagnetic properties of ferritin and hemosiderin, the major storage forms of iron, to

determine tissue iron concentrations. The magnetic susceptibility is determined by the strength and direction of the magnetic response of a tissue when a constant magnetic field is applied. Normal human tissue evokes a very weak magnetic response that is opposite to the applied field. Hepatic tissue with iron overload produces a response in the same direction as the applied magnetic field, and the strength of the response is proportional to the iron concentration in the tissue (113). Hepatic iron estimations by this method have been shown to closely correlate with tissue iron estimations by liver biopsy (114). A number of studies have used SQUID to assess the response to chelation therapy with deferiprone and desferoxamine in patients with β-thalassemia (115,116). The widespread use of SQUID is limited by the cost and limited availability of the equipment for the test. Only a few susceptometers were in clinical use, as of 2005.

Other noninvasive tools for assessing hepatic iron content have been described. One technique that utilizes a modification of MRI, measuring tissue proton transverse relaxation rates, and allowing liver iron concentration measurements (117). Advantages of this technique are avoidance of motion induced artifact in hepatic iron assessment with T2-weighted images (118).

Magnetic resonance imaging for iron overload: conclusion

Although liver biopsy remains the "gold standard" for the diagnosis of hepatic iron overload and the assessment of hepatic fibrosis, there are several clinical scenarios in which MRI can be useful, including the following: (i) Patients without the genotype for hereditary hemochromatosis (HHC) in whom there is nevertheless a suggestion on laboratory testing of possible excessive iron stores, making it desirable to exclude iron overload. For example, patients with chronic hepatitis C or fatty liver disease often have elevated ferritin levels, but may have mild or no secondary iron overload and therefore, do not require iron depletion therapy; (ii) patients with the genotype for HHC, that is, homozygosity for the C282Y gene but are unlikely to have advanced fibrosis, such as patients under 40 years of age with ferritin less than 1,000 ng/mL; (iii) patients with the genotype for HHC and cirrhosis in whom liver biopsy is risky because of coagulopathy, and in whom an MRI can confirm the presence of iron overload.

Hepatic Venous Thrombosis

Hepatic venous thrombosis (HVT) is characterized by hepatic venous outflow obstruction at the level of the inferior vena cava (IVC), hepatic veins, and/or the small centrilobular venules (veno-occlusive disease [VOD]). HVT occurs as a consequence of a variety of congenital and acquired hypercoagulable states including myeloproliferative disorders, paroxysmal nocturnal hemoglobinuria, antiphospholipid syndrome, use of oral contraceptives, and deficiencies of protein C, protein S, and antithrombin III. Other associated conditions include inferior vena caval membranous webs (seen more commonly in Asia), inflammatory bowel disease, and trauma; it is estimated that up to 60% of cases are idiopathic. The condition may have fulminant, acute, subacute, or chronic presentations and should be suspected in patients presenting with ascites, abdominal pain, and hepatomegaly.

The diagnosis of VOD is difficult based on radiological imaging alone, and often requires a liver biopsy. This discussion will focus on the diagnosis of thrombosis of the IVC and hepatic veins. Imaging may demonstrate the obstruction of hepatic venous or IVC blood flow. Furthermore, changes in the liver parenchyma occurring as a consequence of HVT may also be seen. Other findings include caudate lobe hypertrophy (which has independent venous drainage), ascites, and splenomegaly.

Ultrasonography

Ultrasonography with color Doppler imaging is an excellent first-line imaging technique for the diagnosis of hepatic vein thrombosis. Characteristic findings of hepatic vein thrombosis on ultrasound include absence, reversal, or turbulence of flow in a large hepatic vein; large intrahepatic or subcapsular collateral vessels; a "spider-web" appearance near the hepatic vein ostia without a normal appearing hepatic vein in the vicinity; and an absent or flat hepatic vein waveform (119,120). The presence of collateral vessels is more sensitive than alterations in hepatic venous flow, which may be seen in association with cirrhosis from other causes as well. Membranous webs may appear as an echogenic obstruction on ultrasound (121).

Doppler ultrasonography provides useful information on the flow direction and pattern through the hepatic veins and IVC. With HVT, blood flow may be noted to be absent, slowed, reversed, turbulent, or without pulsatility. Intrahepatic or subcapsular collateral vessels with hepatofugal flow (away from the liver), is a sensitive finding for HVT. The sensitivity of Doppler ultrasonography has been reported to be greater than 85% (120,122).

Changes in the liver parenchyma occurring as a consequence of venous outflow obstruction may be seen, although these findings are less specific. These changes include hypoechoic areas representing hemorrhagic infarcts in patients with acute forms of the disease, and increased echogenicity in patients with

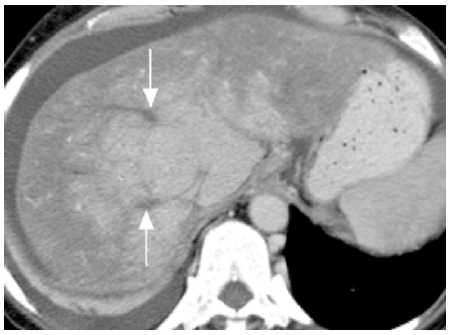
chronic HVT who have developed cirrhosis. The caudate lobe may appear enlarged; however, this may also be seen in patients with cirrhosis from other causes. The reported accuracy of ultrasound imaging for the diagnosis of HVT is 70% (119). Ultrasound is inexpensive and widely available, but is operator dependent and its usefulness is limited in obese patients.

Computed tomography

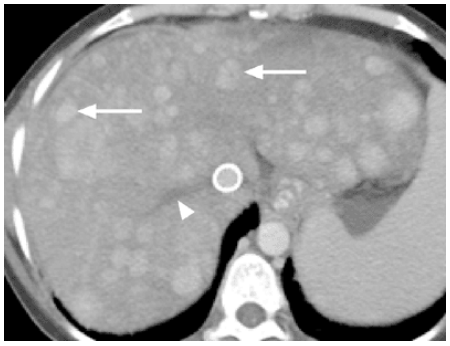
CT scan is helpful for the evaluation of parenchymal abnormalities, caudate lobe enlargement, and the presence of collateral vessels (119). The peripheral atrophic liver parenchyma may demonstrate more enhancement than normal or hypertrophied liver. On dual-phase CT scan, the central liver initially appears hyperdense and then in the washout phase, appears hypodense in comparison with the peripheral liver tissue, which gradually accumulates contrast via subcapsular collaterals (Figs. 4.21 and 4.22). This appearance has been described as a "flip flop" pattern. The presence of portal hypertension may cause delayed enhancement of hepatic veins. As a result, unopacified hepatic veins may be confused with HVT (123). This results in a decreased accuracy of CT scan for the diagnosis of HVT which has been reported to be as low as 50% in some studies (119).

Magnetic resonance imaging

Advantages of MRI include superior visualization of the IVC and hepatic veins, as well as the liver parenchyma. Furthermore, MRI may allow differentiation between the acute and chronic forms of the disease. Differences in signal intensity between the peripheral and central areas of the liver and differences in enhancement patterns have been described in patients with acute versus chronic HVT. In acute HVT, peripheral hepatic signal intensity in T1- and T2-weighted images is lower and higher, respectively, than the central liver (124,125). In contrast, the differences in signal intensity are less pronounced in patients with chronic HVT. Similarly, gadolinium-enhanced images demonstrate diminished peripheral enhancement in acute HVT but minimal differences in enhancement in patients with chronic HVT. The caudate lobe may be spared the effects of the disease in the early stages of the process due to its independent venous drainage, and may therefore have a normal or increased enhancement. Patients with acute on chronic and subacute presentations have MRI findings in between the extremes just described. In chronic HVT, nodular regenerative hyperplasia may develop, appearing as large nodules with increased signal intensity on T1-weighted images. Other findings in chronic HVT include intrahepatic collateral vessels which appear as "comma-shaped" structures (126) and a "spider-web" appearance of collateral vessels (124). Accuracy rates of 80% have been reported for detection of HVT by MRI. (119)



• **Figure 4.21** Acute Budd-Chiari syndrome. Portal venous phase contrast-enhanced computed tomographic scan shows peripheral low attenuation of the liver parenchyma, sparing of the caudate lobe (in which attenuation is normal), ascites, and thrombosed hepatic veins (*arrows*).



• **Figure 4.22** Chronic Budd-Chiari syndrome with nodular regenerative hyperplasia. Portal venous phase contrast-enhanced computed tomographic scan shows multiple hyperattenuating nodules (*arrows*). Note the caval stent for treatment of prior inferior vena cava occlusion and thrombosed right hepatic vein (*arrowhead*).

Venography

Hepatic venography, performed by inferior vena caval opacification and retrograde hepatic vein cannulation via access through the femoral vein, allows the direct assessment of hepatic vein and IVC patency. Characteristic patterns on retrograde hepatic venography in patients

with HVT include a "spider-web" pattern when the catheter tip is wedged in an occluded vein, a network of collateral veins starting at the catheter tip and

ending near the entry of the hepatic vein into the IVC in patients with a partially occluded vein, and a patent hepatic vein proximal to a stricture (127,128). Venography allows the measurement of the gradient between the portal vein and the IVC to determine the feasibility of portacaval shunting. There is a greater risk of complications with hepatic venography compared to other imaging modalities given its more invasive nature.

Summary

Ultrasonography with Doppler imaging should be the first test when Budd-Chiari syndrome is suspected. An MRI should be considered next. Although CT scan allows better visualization of the hepatic parenchyma and detection of collateral vessels, its use is limited by inaccuracy when hepatic veins are not visualized, and should be considered in patients who cannot undergo MRI. Venography, now used uncommonly, should be reserved for patients in whom the diagnosis cannot be confirmed by other radiologic imaging modalities, for patients in whom transjugular intrahepatic portosystemic shunt (TIPS) placement is planned, or for patients in whom the venous anatomy needs to be defined prior to surgery.

Hepatic Cysts

Liver cysts are often found incidentally when abdominal imaging is done for other indications. Liver cysts may either be single or multiple and varied in size, and their prevalence tends to increase with age. Solitary hepatic cysts have been reported to occur in 3% to 18% of patients (129,130,131). Differences in the reported prevalence of hepatic cysts may be attributed to the population screened and the method used. Solitary cysts may be infectious, neoplastic, or traumatic in nature, or may be the result of congenital malformations. Most cysts are asymptomatic and do not require therapy. However, large cysts may cause abdominal symptoms including right upper quadrant pain, abdominal distension, bloating, and anorexia. Such large, symptomatic cysts may require drainage either by percutaneous or surgical techniques. Rarely, bleeding may occur into cysts or they may become infected.

Most hepatic cysts are benign. Neoplastic cysts include biliary cystadenomas and cystadenocarcinomas. Multiple liver cysts may be seen in patients with polycystic liver disease. With age, these cysts increase in number and size and may be associated with complications including hemorrhage, infection, rupture, biliary obstruction, portal hypertension, and malignancy (132).

Characterization of the cysts by a variety of imaging modalities allows the important differentiation between benign and neoplastic cysts.

Ultrasonography

Ultrasonography allows differentiation between cystic and solid lesions. Simple cysts in the liver appear anechoic, with a smooth round contour, and have a thin wall (Fig. 4.23). Echinococcal cysts have septations and may demonstrate cyst wall calcifications; the presence of daughter cysts on an ultrasound helps in the diagnosis of these lesions. Other cyst features that can be assessed by ultrasound include the presence or absence of septations and debris within the cyst (Fig. 4.24). Biliary cystadenomas have septae and are multilocular. Septae or nodularity within the cyst are suggestive of malignancy. Hemorrhage into a cyst may make the discrimination between simple and malignant cysts difficult.

Computed tomography

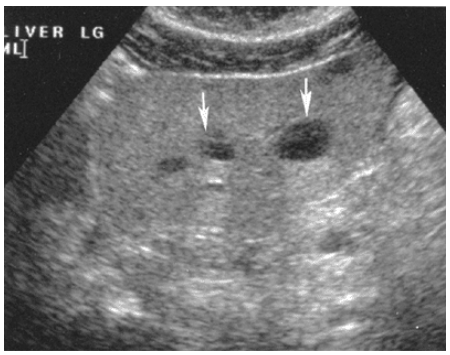
For the evaluation of hepatic cysts, CT scan should be done with and without intravenous contrast. On CT scan, hepatic cysts do not enhance after the administration of IV contrast and demonstrate water attenuation (0 to 10 Hounsfield units). Furthermore, cyst walls are usually imperceptible. CT scan is useful in providing data about the relationship between a cyst and its surrounding liver parenchyma and vascular and biliary structures.

Septae or papillary projections within a cyst are features suggestive of neoplasia. After the administration of intravenous contrast, the cyst walls, septae, and soft tissue components of biliary cystadenomas enhance.

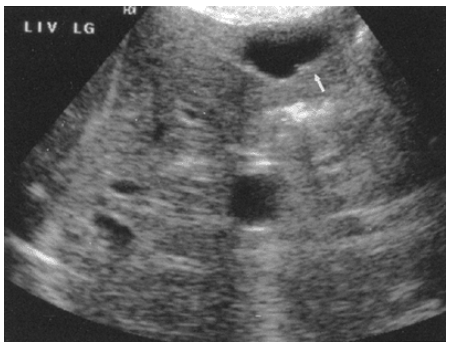
Magnetic resonance imaging

On MRI, simple hepatic cysts demonstrate the same signal intensity as water, that of a high signal intensity

on T2-weighted images and low signal intensity on T1-weighted images. They do not enhance after the administration of gadolinium (Fig. 4.25). The presence of enhancing components post gadolinium indicates a complex cyst, which may be of an infectious, inflammatory, or malignant etiology.



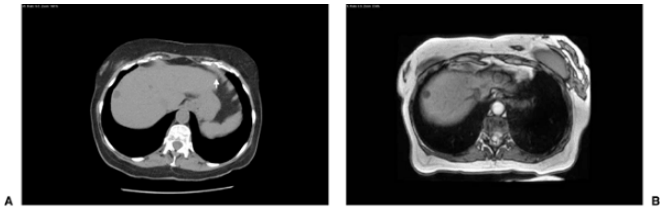
• **Figure 4.23** Liver cysts. Longitudinal ultrasound image shows two simple hepatic cysts (*arrows*) with posterior acoustic enhancement.



• **Figure 4.24** Complex hepatic cyst. Longitudinal ultrasound image shows anterior hepatic cyst with wall irregularity (*arrow*) that suggests complex hepatic cyst.

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is discussed in detail in Chapter 23. The issues involving imaging in clinical practice are as follows: (i) Does MRCP suffice in the diagnosis of PSC, rendering endoscopic retrograde cholangiopancreatography (ERCP) unnecessary in certain clinical situations, and (ii) what is the role, if any, of periodic imaging, potentially combined with tumor marker surveillance, in the early detection of cholangiocarcinoma (CCA) given the substantial lifetime risk of 6% to 20% of this complication in patients with PSC (133,134).

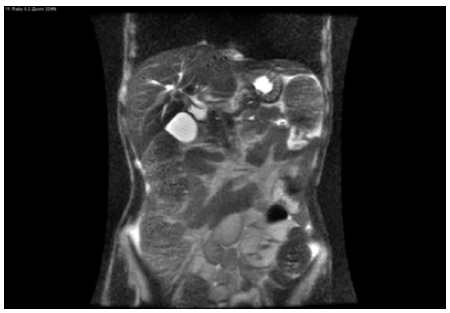


• **Figure 4.25** Liver cysts. **A:** Computed tomographic image demonstrates a 1.4 ×1.3 cm hepatic cyst (*arrow*) in the anterior segment of the right lobe. The lesion is nonenhancing and demonstrates water attenuation. **B:** Magnetic resonance image demonstrates the same cyst (*arrow*).

Studies have shown the sensitivity and specificity of MRCP in the diagnosis of PSC to be excellent, approaching the corresponding values for ERCP. An advantage of MRCP is that it allows the visualization of all ducts, including those proximal to severe stenoses, which may not be seen on ERCP. In a study in which two radiologists independently conducted a blinded random review of MRCP in 102 patients, of whom 34 had PSC, interobserver agreement was excellent and the higher scoring radiologist detected PSC with a sensitivity of 88%, specificity 97%, positive predictive value 94%, and negative predictive value 94% (135). Consistent with these results, many clinicians including the authors have had the experience of seeing occasional patients whose MRCP studies have been read as negative in whom ERCP has revealed characteristic features of the diagnosis. This may be particularly expected in patients with early intrahepatic disease alone. Indeed, two caveats also apply to ERCP. First, the diagnosis can be missed if inadequate filling pressures are used to study the intrahepatic biliary tree, and second, some patients with early PSC may not have any findings even on a technically well done ERCP. In such patients, liver biopsy may reveal characteristic changes such as "pericholangitis" and periductal fibrosis prior to the development of large ductal changes. Thus, even ERCP does not have 100% negative predictive value for this disease.

A more common scenario is the finding of characteristic changes of PSC on MRCP studies (Fig. 4.26), at which time the clinician must decide whether to still obtain an ERCP with its attendant risks. Potential arguments in favor of this approach are that ERCP has long been the "conventional" means of ascertaining the diagnosis, that ERCP will establish the baseline biliary anatomy with the highest degree of accuracy for the purposes of future comparison, and that ERCP

will afford the opportunity for cytologic brushings in the event that dominant strictures are encountered. Since MRCP can be overwhelmingly suggestive of the diagnosis, however, the first of these arguments is not compelling. Moreover, since MRCP can delineate the anatomy well, one can argue that a high quality baseline MRCP can serve as an adequate basis for future comparison. Finally, there are no guidelines indicating that baseline brush cytologies should be obtained when PSC is first diagnosed in the absence of suspicious clinical changes, for example, worsening cholestasis, recurrent jaundice or cholangitis, or the absence of a dominant stricture. Therefore, in a stable patient with features of PSC but without recent change in clinical or biochemical status, a diagnostic MRCP is sufficient to establish the diagnosis of PSC firmly, and the patient can then be followed.



• **Figure 4.26** Primary sclerosing cholangitis. Magnetic resonance cholangiopancreatography image shows multifocal dilatation (*thin arrow*) and narrowing (*thick arrow*) of the bile ducts.

An effective screening regimen to detect cholangiocarcinoma at a treatable stage is a coveted goal in patients with PSC. At the present time, there is no such regimen of proven efficacy, and clinicians vary in their practices from no screening to schedules of periodic determination of tumor markers and/or imaging, albeit in the absence of evidence that detection will result in altered outcomes. The use of the tumor markers CA-19-9 and carcinoembryonic antigen (CEA) was supported by a study of 74 patients with PSC, of whom 15 had tumors, 22 had severe PSC necessitating transplantation with nonmalignant explants, and 37 had stable PSC. An index using the formula CA-19-9 + (CEA × 40) with a cutoff of 400 gave an accuracy of 86% in the diagnosis of cholangiocarcinoma, with 10 of the 15 cases of cancer having an increased value compared with none of a group of 22 cases with no tumor. Six of eleven patients with occult tumors had abnormal values (136). In another multicenter, case-control study, 26 patients with PSC and CCA diagnosed over a 7-year period at eight academic centers were compared with 87 patients with PSC but no CCA. Serum CA-19-9 was significantly higher in patients with CCA than in those without (mean 177 U/mL vs. 61 U/mL). A serum CA-19-9 greater than 100 U/mL had 75% sensitivity and 80% specificity in identifying patients with PSC and CCA (137).

On the other hand, a clinical benefit could not be demonstrated in a prospective study of 75 patients with PSC without clinical signs of CCA investigated every 6 months for 3 years with liver tests and four tumor markers—CEA, CA-19-9, CA 50, and CA 242. The patients were then followed clinically for another 5 years. The authors found the tumor markers to be of limited value for the detection of CCA in patients with PSC because of low specificity (138). A previous study examining explants in patients coming to liver transplantation similarly found that CA-19-9 levels were not predictive of CCA (139).

There are no prospective studies with long-term outcomes combining tumor markers with imaging studies. Of the conventional imaging studies, the one that might in theory be most suited to screening is MRI, because the liver parenchyma can be visualized with T1-weighted images, while the biliary tract can be outlined with T2-weighted images. Thus, MRI-based screening can potentially detect peripheral cholangiocarcinomas as well as masses arising from the main biliary tree, or at least document changes such as the development of dominant strictures in the biliary tract, which might require further investigation. Again, however, there are no studies indicating that imaging surveillance affects outcomes.

There has been considerable interest in PET scanning for the detection and staging of CCA, whether in PSC or otherwise. PET scanning relies upon the fact that malignant tumors have high glucose metabolic rates and accumulate (18F)-fluorodeoxyglucose (FDG), a positron-emitting tracer. In one of the first studies demonstrating promise for PET, six of six patients with known PSC and CCA had "hot spots," while nine patients with PSC and no evidence of CCA and five controls had no such spots (140). A subsequent study that suggested that PET was highly sensitive and specific in a group of 26 patients with CCA, 8 with benign biliary tract disease, and 20 controls, reinforced the implications of this study. The only significant limitation of PET in this study was greater sensitivity for distant metastases than regional lymph node metastases (141).

More recent studies have raised concerns about the sensitivity and/or specificity of PET scanning in the diagnosis of CCA. In a study of 36 patients evaluated for suspected CCA, of whom 31 ultimately proved to have the disease, sensitivity was 85% for the nodular morphology but only 18% for the infiltrating morphology. One patient with PSC and acute cholangitis had a false-positive result, while 7

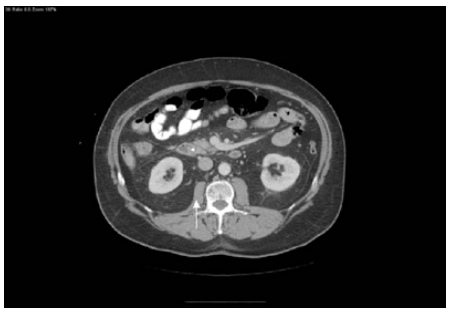
of 12 patients had FDG uptake along the tract of a biliary stent (142). In another study of ten patients with PSC being evaluated for liver transplantation, eight of whom were transplanted and in whom the explants were therefore available for study, there were two false-positive and one false-negative PET scans (143). In another series of 15 patients with suspected hilar tumors, PET was true-positive in 10, false-positive in 2, and false-negative in 3 (144).

The literature on PET scanning does not directly address the issue of surveillance in patients with PSC. Most of the literature addresses the issue of sensitivity and specificity in patients with clinical features leading to the suspicion of CCA, or patients with actual CCA proven by other means in whom correlations with PET were evaluated. In these latter populations PET scanning, although promising, has insufficient accuracy to be used as a stand-alone study, particularly in surveillance, especially with the demonstrated potential for false-positive examinations. Accordingly, as for MRI there are no guidelines calling for the routine use of PET scanning in the screening or surveillance of patients with PSC for CCA. The role of PET scanning requires further study in this population.

Choledocholithiasis

Prior to the widespread availability of MRCP and EUS, noninvasive imaging of the biliary tract centered on sonography and CT scanning. When biliary tract pathology was suspected, direct cholangiography with ERCP or percutaneous transhepatic cholangiography (PTC), with their attendant risks, was required. In the context of suspected choledocholithiasis, the frequent need to proceed to ERCP for its potential therapeutic applications was particularly problematic in the absence of a high level of confidence that the procedure would reveal the underlying pathology in question. Thus, patients were commonly exposed to the risk of ERCP, most notably acute pancreatitis, when in retrospect the procedure would not have been necessary if highly accurate noninvasive imaging were available. As a result, a large body of literature emerged addressing the issue of the proper role for ERCP in suspected, but unproven, choledocholithiasis (145,146).

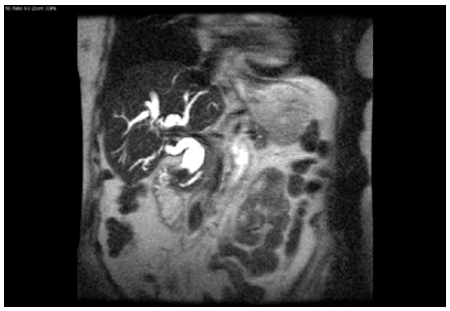
CT scan can detect a substantial proportion of CBD stones, but magnetic resonance cholangiopancreatography and endoscopic ultrasound have dramatically improved upon the ability to make the diagnosis of biliary tract stones without resorting to invasive techniques (Figs. 4.27, 4.28 and 4.29). For common bile duct stones, MRCP has an overall sensitivity of 85% to 100%, with positive and negative predictive values as high as 95% (147,148,149,150,151,152). The chief limitation is in patients with small stones. False negative studies can occur if MRCP slice thickness is greater than 3 to 4 mm, or from motion artifact obscuring small stones if the patients are unable to hold their breath. Pneumobilia and blood clots may mimic small stones. Surgical clips, a tortuous common bile duct, and extraductal compression from the right hepatic or gastroduodenal artery can also cause diagnostic confusion.



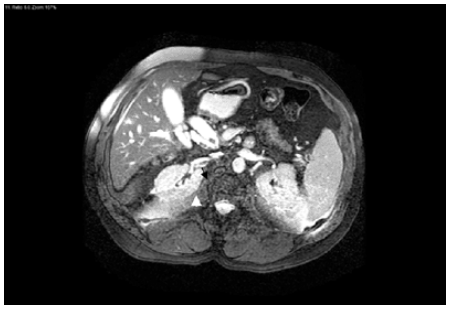
• **Figure 4.27** Choledocholithiasis. Computed tomographic image demonstrating a 5 mm stone in the distal common bile duct (*arrow*).

In addition, MRCP may not always be available within the time frame necessary for it to impact upon therapeutic decision-making, particularly since patients with bile duct stones frequently present with clinical features that require prompt resolution, such as cholangitis or severe pain. Despite these limitations, MRCP has proven to be extremely useful in patients with low or intermediate levels of probability of harboring bile duct stones, and ERCP can often be avoided as a result of negative MRCP examinations in such patients. In such situations, when the gallbladder is intact, the surgeon may proceed to cholecystectomy with a high

level of assurance that preoperative ERCP is unnecessary and that postoperative complications related to unsuspected bile duct stones are unlikely to occur. In such instances, the decision to perform intraoperative cholangiography must be individualized.



• **Figure 4.28** Choledocholithiasis. Magnetic resonance cholangiopancreatography coronal image demonstrating a 1.4 cm stone (*arrow*) in the common bile duct with proximal intrahepatic and extrahepatic biliary dilatation.

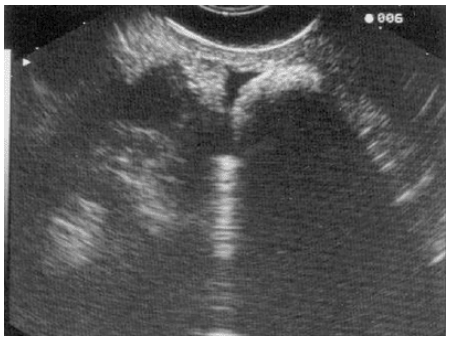


• **Figure 4.29** Choledocholithiasis. Magnetic resonance cholangiopancreatography image showing multiple stones in the common bile duct (*black arrow*) and cyst duct (*white arrow*) with intrahepatic and extrahepatic biliary ductal dilatation.

In contrast, when there is a high level of probability of choledolithiasis, such as rising cholestatic liver chemistries with a dilated bile duct, or active cholangitis, the clinician's judgement may still be that ERCP in the absence of noninvasively demonstrable bile duct stones is desirable. If a patient has presented with recurrent fever and shaking chills over the course of a few days, and has hyperbilirubinemia with a significantly elevated alanine aminotransferase (ALT) and alkaline phosphatase, even a nondilated duct on ultrasound and a negative MRCP may still be associated with the finding of small stones and/or dense sludge emerging from the bile duct after a sphincterotomy. A decision to perform sphincterotomy empirically in such situations must be individualized and based on the level of suspicion for intraductal pathology.

Endoscopic ultrasound has a level of sensitivity and specificity for common bile duct stones equaling or often exceeding that of MRCP, and easily rivaling published figures for ERCP in comparative studies (153,154,155,156) (Fig. 4.30). In one study, for example, EUS had a sensitivity of 98%, a specificity of 99%, a positive predictive value of 99%, a negative predictive value of 98%, and an accuracy of 97% (154). Its major limitation relative to ERCP is that, despite being unassociated with the significant element of risk of ERCP, particularly with regard to pancreatitis, it is still an invasive procedure requiring intravenous sedation. Moreover, the ability to offer it promptly may be limited in many centers, and the expertise to offer it concurrent with ERCP by the same endoscopist within one facility does not exist at many centers. When such expertise does exist, EUS offers a potentially powerful approach, especially in the setting of acute illness such as gallstone pancreatitis, because the diagnosis can be obtained with minimal risk and therapy offered, if appropriate, in one endoscopic session (157). Recently, intraductal ultrasound, which can also be done concurrent with ERCP, has been shown to be capable of increasing

the yield over cholangiography alone, and can also distinguish air bubbles introduced during cholangiography from true stones (150,158).



• **Figure 4.30** Choledocholithiasis. A endoscopic ultrasound image demonstrating a stone in the common bile duct with distal acoustic shadowing (*arrow*).

Ultrasound Guidance for Liver Biopsy

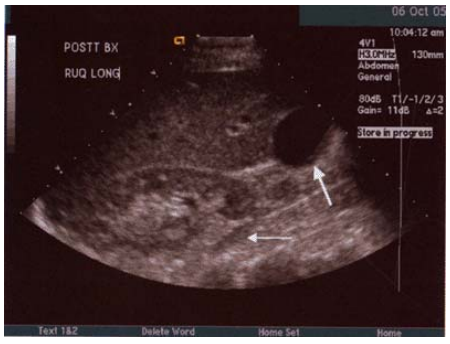
Liver biopsy is an essential part of the diagnostic evaluation of many parenchymal liver diseases. The determination of the degree of inflammation and fibrosis in patients with chronic hepatitis C is probably the most common indication for this procedure, but other diseases for which the procedure is frequently performed include hepatitis B, fatty liver disease, autoimmune hepatitis, cholestatic liver diseases, and the investigation of elevated liver tests. Most liver biopsies are done percutaneously via a lateral intercostal approach, although an anterior subcostal percutaneous approach to the left lobe of the liver has also been described (159).

Complications associated with percutaneous liver biopsy can be divided into minor and major events. Minor complications include pain at the biopsy site or right shoulder, transient hypotension, and skin hematomas. Major complications, usually related to the inadvertent puncture of organs in close proximity to the liver, include bleeding with hemoperitoneum or hemobilia, bile peritonitis from perforation of the gallbladder, puncture of the lung resulting in hemothorax, pneumothorax or pleural effusion, and puncture

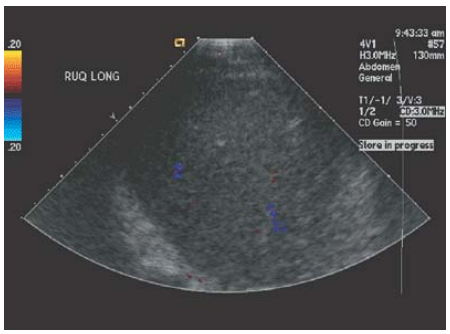
of the colon, pancreas or right kidney. Punctures of other organs do not invariably result in significant clinical problems, however. Based on a published series of thousands of liver biopsies, complication rates ranging from 0.3% to 3.2% (160,161,162) and mortality rates of 0.01% to 0.12% (161,163) have been reported.

In an effort to improve patient safety and to decrease the rate of complications, prebiopsy ultrasonography to delineate a patient's anatomy and choose the optimal site for the biopsy is widely performed. The role of routine ultrasound guidance in the performance of biopsy of a focal hepatic lesion is well established (164) but remains controversial for diffuse liver diseases with proponents both for (165,166,167,168,169,170) and against (171,172) its use. Although there are limited data supporting an improved outcome with this practice, some practitioners have incorporated ultrasound guidance so firmly into their method of performing liver biopsies that they feel that it would be unethical to do a randomized controlled trial comparing the impact of ultrasound-guided versus blind liver biopsy (169).

Bedside ultrasonography prior to percutaneous liver biopsy is used either after or in place of localization of the biopsy site by percussion. Less commonly, real-time ultrasonography is done with visualization of the liver biopsy needle throughout the procedure. Ultrasound allows the identification and avoidance of perihepatic structures including the gallbladder, right kidney, colon, and the right lung (Figs. 4.31 and 4.32). Ultrasonography also allows evaluation of large blood vessels. Riley et al. conducted a prospective study of 165 consecutive outpatient liver biopsies where the site for the biopsy was first identified using percussion, following which ultrasonography was done to evaluate the adequacy of the marked site. Ultrasonography resulted in either an alteration of the site of biopsy or aborting the procedure in 25 (15.1%) of the patients. The reasons for the change in management included the identification of lung, gallbladder, large blood vessels, colon, a greater than 4 cm rim of ascites, or a focal liver lesion in the "field" of biopsy. No complications occurred in this study (173).



• **Figure 4.31** Ultrasound image demonstrating the gallbladder and kidney in the liver biopsy field (*arrows*).



• **Figure 4.32** Ultrasound demonstrating a good window for percutaneous liver biopsy.

Image guidance may be especially useful in obese patients, those with chronic obstructive pulmonary airway disease or ascites, and in patients in whom there was failure of localization by percussion. Some centers also perform a postprocedure ultrasound with Doppler for the early identification of potential complications, especially bleeding. When this occurs, blood may be seen extending from the intrahepatic vessel to the capsule or a subcapsular hematoma. The disadvantages of ultrasonography include the extra time and cost added to the procedure. Furthermore, many gastroenterologists are not trained in ultrasonography requiring the presence of an ultrasonographer or technician for the procedure.

Studies have surveyed the practices of hepatologists in the United States, England, and France (167,168,174,175). A survey of practitioners in the Washington DC metropolitan area (168) revealed that 64% of gastroenterologists utilized image guidance for liver biopsies. All of these physicians used a radiologist to perform the ultrasound. A second survey of practices of 128 American Association for the Study of Liver Disease members revealed that although 76% of respondents were taught the blind technique of liver biopsy during fellowship training, 76% were currently using ultrasound guidance (175). Most gastroenterologists (97%) did not routinely perform a postbiopsy ultrasound. A survey of liver biopsy practices in the United Kingdom (174) found that 34% of biopsies were done with image guidance although some of the biopsies included in this study were done by radiologists for focal liver lesions. Two deaths were reported in this

series of 1,500 biopsies (intraperitoneal hemorrhage, bile peritonitis from gallbladder perforation); both occurred in patients who underwent blind biopsies. A prospective review of 2,084 liver biopsies in France reported that ultrasound guidance was used by 56% of gastroenterologists (167). In this study, complications with puncture of other viscera occurred only with blind biopsies. The American Gastroenterology Association published a "Statement on outpatient percutaneous liver biopsy" prepared by its Patient Care Committee (176). They noted that the procedure appears to be safer when performed with ultrasound guidance, but did not include ultrasound guidance in their recommendations.

There are limited data comparing ultrasound-guided with blind liver biopsies. Most of the data are retrospective in nature or have other methodological weaknesses that limit their generalizability. Caturelli et al. performed a retrospective review of 753 consecutive percutaneous liver biopsies done with ultrasound guidance (170). A retrospective control group of 702 patients who had undergone blind liver biopsies was used for comparison. A significantly higher major complication rate was noted in the historical cohort compared to the patients undergoing image-guided biopsy (2.1% vs. 0.53%, $P = 0.007$). Also, prebiopsy ultrasound increased the diagnostic yield of the procedure. Colombo et al. randomized 1,192 patients with diffuse liver disease to undergo a liver biopsy with either a Tru-Cut or a Menghini needle (169). All the patients underwent a prebiopsy ultrasonography. A low rate of multiple passes and failed procedures in this study was attributed to the routine use of image guidance. A prospective study compared patients undergoing blind liver biopsies using a Tru-Cut needle with ultrasound-guided biopsies using Automated-Needles (165). Patients undergoing guided biopsies had significantly less pain and morbidity after the procedure, than those undergoing a blind procedure. However, it is difficult to attribute this difference to the ultrasound guidance alone, given that the type of needle used was different in the two groups. Papini et al. compared the standard blind Menghini liver biopsy technique with an ultrasound-guided anterior subcostal approach to the left lobe of the liver (159). Patients undergoing the ultrasound-guided technique had fewer complications and better sample sizes. The benefits of ultrasound guidance suggested by this study are limited by the fact that the anterior subcostal approach is not commonly performed, the biopsies were done primarily by radiologists, and the biopsy technique differed in the two groups.

The single randomized controlled trial comparing blind versus guided liver biopsies was conducted by Lindor et al. who compared the use of Tru-Cut versus automatic-needle biopsy with or without the use of ultrasonography (177). Eight hundred thirty-six patients were enrolled in this study, most of whom had chronic viral hepatitis. The use of ultrasonography was found to result in significantly lower rates of hospitalization (2.2% vs. 0.5%, $P = 0.04$), and significantly less pain requiring treatment (50% vs. 37%, $P = 0.03$). Eleven patients required hospitalization for pain (seven patients) or hypotension (four patients); nine patients underwent a blind liver biopsy, and two of these had ultrasound guidance. However, there was no significant difference in the frequency of bleeding and hypotension between the two groups. Furthermore, investigators were aware of whether patients underwent ultrasonography or not, and there were no formal objectives for determining the occurrence of pain or hypotension after the procedure, raising the possibility of bias in this study. One major complication occurred in a patient who sustained a gallbladder perforation; this patient underwent a blind biopsy.

A number of studies have suggested that ultrasound guidance may result in less abdominal pain after the procedure. Proposed mechanisms for this include avoidance of the pleura and large blood vessels and bile ducts; puncture of these structures may cause pleural irritation, subcapsular bleeding, or irritation of the diaphragm by bile or blood. **A**lso, ultrasound guidance may allow the gastroenterologist to feel more comfortable with the site chosen for the biopsy, leading to less probing of the intercostals spaces and liver capsule with the biopsy needle.

Two studies have assessed the cost-effectiveness of ultrasound guidance for percutaneous liver biopsy (178,179). In a decision analysis model, ultrasonography was found to be cost-effective if the additional charge for the ultrasound was low (<$102) (178). Another study, based on the assumption that ultrasonography reduces the incidence of complications from percutaneous liver biopsy by 60%, found that ultrasound-guided liver biopsy is cost-effective.

Conclusion

There has been a strong trend toward the use of ultrasound guidance as an adjunct to the performance of percutaneous liver biopsy in patients with nonfocal liver disease. To an important extent, in the United States this has been driven by a shift in many gastroenterology practices toward the performance of the procedure by radiologists. Among hepatologists and gastroenterologists who perform the procedure, an increasing number also use ultrasound guidance for the reasons outlined in this section. Several published reports suggest that there may be less postprocedure pain and other complications when ultrasound guidance is used. However, the cumulative published experience is not sufficiently conclusive to establish a uniform standard.

The Hepatic "Incidentaloma"

The expanding use of cross-sectional imaging techniques has led to the increased detection of hepatic lesions in asymptomatic patients. This section aims to provide an over all guide of what to do with these patients and their appropriate workup. The general starting point here is that the lesion is seen on a routine ultrasound in an asymptomatic patient and the patient is then referred for further evaluation.

Clinical history and examination

The first step involves a routine clinical history and examination. The age, sex of patient, history of oral contraceptive use, occupational history, personal and family medical history should all be recorded. The clinical examination aims to identify any stigmata of liver disease or other systemic diseases. If either of the above point to an underlying disease, then evaluation will change to focus on that. The following discussion only applies to the asymptomatic patient. This discussion will use the ultrasound report as a triage point from which to guide us to the next appropriate imaging test.

The ultrasound report should indicate whether this lesion is solid or cystic. It should also indicate if this is a simple or complex cystic mass.

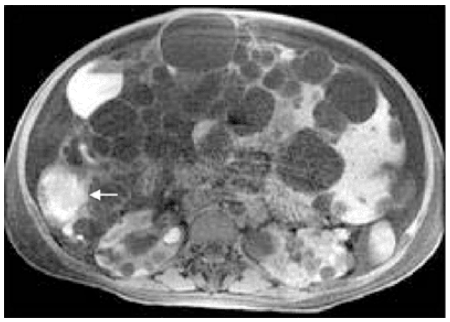
Simple cyst

If this is a solitary simple cyst then there is no need to do anything else except to reassure the patient. Criteria defining a simple cyst are discussed in the section devoted to hepatic cysts. Liver cysts occur in 2.5% of the general population. If there are multiple cysts, the next thing to consider is the age of the patient. 7% of patients over 80 years will have hepatic cysts (131). In a young patient, however, it is more unusual to have multiple cysts (Fig. 4.33). Underlying syndromes such as polycystic kidney disease or von Hippel-Lindau must be excluded.

Complicated cyst

A complicated cyst can be seen with hemorrhage, infection and malignancy. A history of foreign travel or fever points toward infection, including echinococcus. Simple hemorrhage will not show any enhancement post contrast on either CT scan or MRI. An MRI can be particularly helpful in assessing complex cystic cases as it can help to age the hemorrhage, and assess for enhancement also with the use of subtraction techniques.

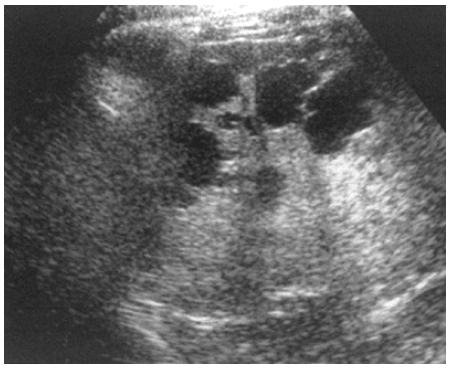
The presence of solid enhancing lesions and nodular or thickened septations raises the questions of malignancy. The most common etiology would be that of metastatic disease from a cystadenocarcinoma usually from the ovary, bowel, or pancreas (Fig. 4.34). Other considerations are a bile duct adenoma or adenocarcinoma. In these cases, percutaneous biopsy of the liver lesions is recommended with ultrasound guidance.



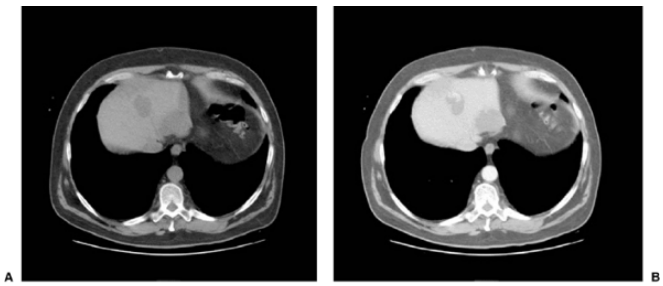
• **Figure 4.33** Axial T1-weighted image shows most of the parenchyma of the liver is replaced with cysts of varying size. The high signal in some of the cysts is secondary to either blood or protein (*arrows*).

Solid lesion

The initial guiding point here will be the description of the echogenicity of the lesion from the ultrasound report. This should determine your next imaging test. The age and sex of the patient should be considered, as the most common benign lesion in the liver is a hemangioma. We shall use this as a starting point.



• **Figure 4.34** Cystic hepatic metastatic lesion. Transverse ultrasound image shows multiple complex cysts in a patient with a history of ovarian carcinoma. The biopsy findings indicated the presence of cystic metastatic disease.



• **Figure 4.35** Hemangioma. Noncontrast **(A)** computed tomographic image showing a nonspecific hypodense lesion measuring 3.5 × 3.0 cm in size (*arrow*). Arterial **(B)** enhanced computed tomographic image demonstrates peripheral nodular enhancement characteristic for a hemangioma (*arrow*).

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Hyperechoic lesion—hemangioma

In young women the most common etiology of an asymptomatic lesion would be a hemangioma. If the description is that of a homogenous hyperechoic lesion which is less than 3 cm with well-defined margins and posterior acoustic enhancement, this suggests a hemangioma (180). In the absence of any other risk factors, it has been advocated that the patient should be managed conservatively with simple follow-up in 6 months and no further follow up if the lesion is stable (181).

If further evaluation is deemed appropriate, then the most useful test to confirm this is a dedicated liver MRI. An MRI is recommended over CT scan because of the absence of radiation in this generally younger female population. Also, the sensitivity and specificity of MRI for hemangiomas has been reported as high as 90% to 98%. The classic imaging finding is that of early peripheral nodular enhancement with delayed (182) (Fig. 4.35). When contrast CT scan is used, delayed scans should be performed at 5 minutes.

Giant or atypical hemangiomas can cause problems in diagnosis as they often contain central areas of fibrosis. Giant hemangiomas are usually defined as those larger than 10 cm (183). In these cases, Tc 99m pertechnetate–labeled red blood cell scintigraphy can play a very useful role. With this method, there is decreased activity on early or perfusion images and increased activity on delayed or blood pool images. The specificity of red blood cell scintigraphy in the detection of hemangioma approaches 100% with very few false-positive results reported (184,185) (Fig. 4.36).

Limitations of this technique are that the sensitivity in the detection of hemangioma varies considerably from (65% to 90%). Larger lesions, which are 2 cm or larger can usually be seen with planar imaging. However, as lesion size decreases below 1.5 cm, they are difficult to detect. Lesions which are deep within the liver or adjacent to large vascular structures are less often identified even with single photon emission CT scan (186,187). Therefore, radionuclide scintigraphy is a valuable tool when the diagnosis cannot be achieved with other imaging modalities (188).

Percutaneous biopsy, including fine-needle aspiration biopsy, has been described as both safe and effective. It does however carry the risk of hemorrhage (189,190).

Isoechoic lesion—focal nodular hyperplasia

This is the second most common benign liver lesion. It is also more common in younger women aged 30 to 50. It is most often a solitary mass with a central scar (191). Although use of the oral contraceptive pill has not been shown to cause this lesion, it is thought that estrogens may influence its growth and development (192).

This diagnosis should be considered, if on ultrasound lesion's echogenicity is similar to that of the adjacent liver parencyhma. This has led to its description as the "stealth lesion." It can be very subtle and difficult to define. This differs from a hemangioma which is generally hyperechoic and easy to distinguish (193). The central scar may be seen as a central hypoechoic linear or stellate area (194).

Therefore, if a lesion is described as "isoechoic" or difficult to define, this should raise the diagnosis of focal nodular hyperplasia (FNH). The correct diagnosis of FNH is critical, as other hypervascular liver lesions such as hepatic adenoma, HCC, and hypervascular

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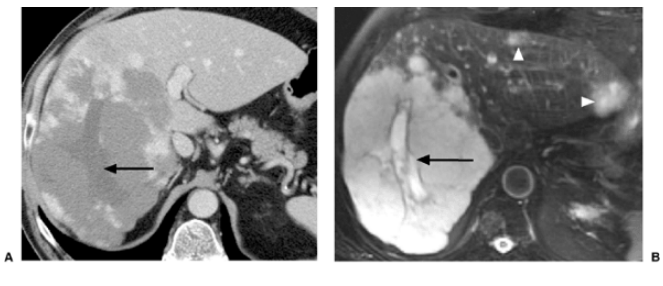
metastases will require treatment. In particular, fibrolamellar carcinoma must be distinguished from FNH, because both are seen in young adults without prior history of cirrhosis, and because both have a central area of scar as a hallmark at imaging.



• **Figure 4.36** Giant hemangioma. **A:** Portal venous phase contrast-enhanced computed tomographic image shows typical peripheral nodular enhancement. Note the lack of enhancement, however, of the central scar (*arrow*). **B:** T2-weighted fast spin-echo magnetic resonance image shows heterogeneous high signal intensity and central scar (*arrow*). Additional smaller hemangiomas (*arrowheads*) are present in the left lobe.

It is suggested that the next test ordered should be an MRI. This will confirm the presence of a mass and also evaluate for other masses, which may have been missed at ultrasound. A preference is expressed by the authors for MRI due to the lack of radiation and the avoidance of iodine based contrast issues in this generally younger population. However, if MRI is not available, CT scan is an excellent alternative. Studies have indicated that MRI has 70% sensitivity and 98% specificity for the diagnosis of FNH (195). Other studies have indicated a

greater degree of difficulty with the diagnosis (196,197). Standard MRI criteria for a typical FNH have been described (198,199).

A classic FNH can generally be diagnosed with confidence on CT scan or MRI. Similar to a hemangioma, its appearance on imaging has been described as classic (80%) and non classic (20%). Generally, on T1-weighted images FNH is iso- or hypointense, on T2-weighted images it is slightly hyper- or isointense, and has a hyperintense central scar on T2-weighted images. It shows intense homogeneous enhancement in the early phase, and enhancement of the central scar in the later sequences (Fig. 4.37).

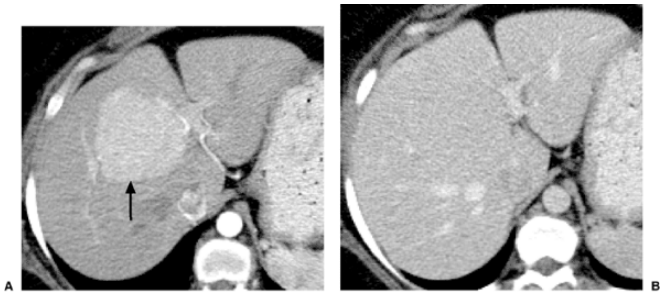
If this diagnosis is made on imaging characteristics alone, it is recommended that a single follow-up examination be performed in 6 to 12 months to document stability (200).

Twenty percent of lesions will remain indeterminate. If FNH is suspected but there are some atypical features, it is suggested that a sulfur colloid scan be performed. FNH is composed of hepatocytes, Kupffer cells, and bile ducts. The reticuloendothelial system component in the liver is the Kupffer cells, which will uptake sulfur colloid. The uptake of Tc 99m–labeled sulfur colloid in substantial quantities at scintigraphy, is highly specific for FNH (201).

Importantly, Kupffer cells are not significantly present in fibrolamellar carcinomas, so they appear on Tc 99m–labeled sulfur colloid images as photopenic defects (197,202,203). Isolated reports have shown that HCC and adenomas can show reticuloendothelial uptake on sulfur colloid scans, but usually to a lesser degree than FNH. However, the majority of adenomas are cold due to an absence of or markedly decreased Kupffer cells (204,205). Hepatobiliary scintigraphy (performed with Tc 99m dimethyl iminodiacetic acid [HIDA] or an analog) can also be useful in diagnosing FNH, as this lesion contains abnormal bile ducts and will appear as a hot spot due to the delayed clearance of the radio isotope.

Hepatocyte selective intravenous agents that are used for liver MRI are distributed in the extracellular space with renal excretion and are taken up by hepatocytes and excreted by the biliary system have shown some promising results. In these cases, the normal information from the post-contrast dynamic scans is available, then a delayed scan is performed. As FNH contains some malformed bile ducts, there is delayed excretion of the contrast agent and typical FNHs have

an iso- or hyperintense appearance relative to the surrounding normal liver, while hepatic adenomas have a hypointense appearance (206,207).



• **Figure 4.37** Focal nodular hyperplasia. Arterial **(A)** and portal venous phase **(B)** contrast-enhanced computed tomographic images demonstrate typical hyperintense, nearly homogeneous arterial phase enhancement (*arrow*) and nearly isoattenuating portal venous enhancement.

Heterogenous or nonspecific appearance—adenoma

Hepatic adenomas are rare benign tumors, and are usually the next consideration in a young female patient. These lesions are less common than FNH. Although the precise pathogenic mechanism of hepatic adenomas is still unknown, they are linked to the use of oral contraceptives, androgen steroid therapy, type I glycogen storage disease, and galactosemia. They are a solitary lesion in 70% to 80% of cases (208).

The sonographic appearance of an adenoma is nonspecific. It can be hyperechoic, hypoechoic, isoechoic, or heterogenous. However, they can be seen as a hyperechoic mass due to the high lipid content of the hepatocytes, or as a result of hemorrhage (209).

In this setting, where the ultrasound is nonspecific, an MRI or CT scan would be a suitable next choice. In most cases, an MRI will be able to exclude a typical hemangioma or FNH. In contrast to an FNH, an adenoma typically shows no central scar and subcapsular feeding arteries. An adenoma is also suggested by areas of fat or hemorrhage (Fig. 4.38). They may be complex in appearance due to repeated hemorrhage (210,211,212,213).

An adenoma is a true neoplasm, which is composed of normal or atypical hepatocytes, and generally lacking Kupffer cells and bile ducts. As noted in the preceding text, Kupffer cells are occasionally present but usually in lower numbers than in the normal liver parenchyma. Bile ducts and portal tracts are absent. Therefore, radionuclide scintigraphy is rarely helpful in the diagnosis of a hepatocellular adenoma (214).

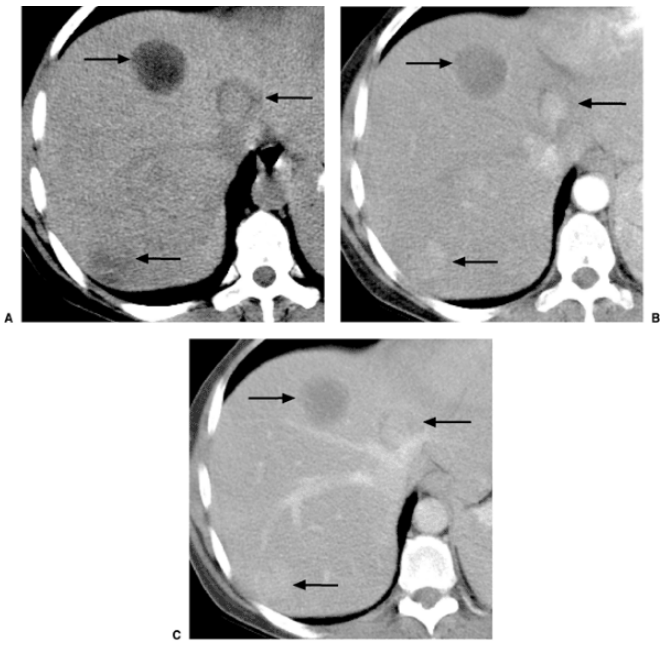
Following the guidelines outlined above, it is usually possible to distinguish an adenoma from an FNH. The other differential to consider is hypervascular metastases. The most common primary in this age group will be from a breast or thyroid primary. Metastases will usually be multiple and will be hypoattenuating or hypointense relative to the normal liver, and areas of fat and hemorrhage are rare in hypervascular metastases. When doubt remains with atypical features present, MRI with hepatocellular-specific contrast agents or nuclear medicine studies can be considered. Ultimately, in indeterminate cases the patient should be referred for an ultrasound-guided biopsy.

Screening for Hepatocellular Carcinoma

HCC is the sixth most common cancer and the third most common cause of cancer-related death worldwide, with approximately 626,000 new cases diagnosed in 2002 (215). In the United States, the incidence of HCC has been increasing with a doubling of the age-adjusted incidence between 1985 and 1998 (215a). The presence of underlying liver disease is a significant risk factor for the development of HCC. The risk is particularly high in cirrhotics, in whom the yearly incidence rate of HCC is 3% (216). Screening for HCC in this at-risk group of patients is widely practiced, and has been endorsed by two consensus conferences on the subject (217,218).

However, many basic questions about the ideal frequency and modality for HCC screening have yet

to be answered, since only one randomized trial has been done to evaluate the impact of the diagnosis of HCC, within versus outside of a screening program, on survival. Furthermore, HCC screening is affected by the patient population that is being screened including the patient race, the underlying liver disease, and the degree of existing liver injury.



• **Figure 4.38** Glycogen storage disease type 1 and multiple hepatocellular adenomas. **A:** Nonenhanced computed tomographic image demonstrates several lesions (*arrows*) containing varying amounts of fatty tissue with lower attenuation than that of the surrounding liver. **B:** Arterial phase contrast-enhanced computed tomography image shows enhancement of the posterior and medial segment adenomas (*arrows*), which are nearly isoattenuating on a portal venous phase image **(C)**.

α-Fetoprotein

Serum α-fetoprotein (AFP) has been reported as a tumor marker for HCC since 1963, but is not specific for HCC, with elevated levels seen in other conditions including chronic liver disease (especially viral hepatitis), and nonhepatic malignancies including pancreatic, gastric and biliary tumors, and germ cell tumors. In patients with HCC, AFP levels may range from normal to greater than 100,000 μg/L, and lack a linear correlation with tumor size or stage. Although AFP levels greater than 400 are highly associated with HCC, not all HCC secrete AFP and elevated levels may be seen in the absence of HCC. Performance characteristics depend on the AFP cutoff used; as the threshold for a "significant" AFP level is increased, the sensitivity of the test decreases and the specificity increases. The trend of serial AFP measurements in a particular patient over

time is perhaps equally, if not more, significant than absolute AFP level in many cases (219).

A number of cross-sectional and retrospective studies have evaluated the performance characteristics of AFP for the detection of HCC (Table 4.2). The sensitivity and specificity, respectively, have been reported for AFP levels of greater than 20 ng/mL (55% to 65%, 80% to 91%), greater than 100 ng/mL (31% to 41%, 97% to 99%), and greater than 200 ng/mL (22% to32%, 99% to 100%).

Performance characteristics of the test determined during prospective HCC screening studies are similar (Table 4.3). The largest studies screened HBsAg positive patients with AFP measurements every 6 months, and reported sensitivity rates of 55% to 64% and specificity rates of 87% to 91% (225,226).

Smaller studies have screened patients with cirrhosis on a 6-monthly basis. AFP cutoffs of 20 ng/mL resulted in sensitivity rates of 39% to 63%, and specificity rates of 76% to 94% (227,228,229,230). A higher AFP cutoff of 100 units resulted in lower sensitivity rates (13% to 41%) and higher specificity rates (93% to 97%) (227,228,230). One study reported performance characteristics of an AFP threshold of 200 with the highest specificity rate of 100% (228).

Other serum markers for HCC including des-γ-carboxy prothrombin (DCP), urinary TGFβ 1, and soluble interleukin-2 receptor have also been proposed and tested for HCC screening, but are not widely used in clinical practice (232,233,234).

Ultrasound

Ultrasound is the most widely used and studied radiologic examination for HCC screening. On ultrasonography, HCC appears as round or oval lesions with sharp, regular borders. There is no specific appearance of HCC on ultrasound and small lesions, in particular, may vary in appearance. Small lesions are often hypoechoic, but may be hyperechoic if there is fat in the lesion. HCC may be indistinguishable from regenerative nodules and, in certain instances, from hemangiomas. The addition of a Doppler examination allows the evaluation of the vascularity of the lesion; unlike regenerative nodules, HCC tends to exhibit neovascularity (a fine network of blood vessels around and extending into the lesion), although this is often difficult to visualize. The presence of a psuedocapsule is very suggestive of HCC but is not often seen.

The use of ultrasonography has been studied for the detection of HCC in studies comparing preoperative ultrasound examinations with a pathologic examination of explanted livers from patients undergoing either liver transplantation or partial hepatectomy. In patients with cirrhosis, sensitivity of ultrasound has varied widely from 30% to 89% (Table 4.4). Greater specificity has been reported in these studies (71% to 100%).

Prospective studies have evaluated ultrasonography every 3 to 12 months for HCC screening and reported higher sensitivity rates (59% to 100%) (Table 4.5) than those reported with AFP screening (13% to 64%). The greatest drawback of ultrasonography for HCC screening is that the test is strongly operator dependent. The test is also affected by the population screened and is not as accurate in patients with cirrhosis, in those who are obese or have significant steatosis, and in differentiating regenerative nodules from HCC.

AFP and Ultrasound

Few studies have compared the effectiveness of this combined screening protocol with either AFP or ultrasound alone. The combination of AFP and ultrasound was compared to AFP alone in a study screening patients with chronic hepatitis B (225). Unfortunately, the sample size of this study was not adequate to compare the two screening protocols. In a mathematical model, it was predicted that AFP and ultrasound

combinations increased the sensitivity of screening by 5% to 10% compared with ultrasound alone (249). In a retrospective analysis of 106 patients who underwent ultrasonography and AFP measurements prior to liver transplantation, the combination of ultrasound and AFP (79%) demonstrated a higher sensitivity than either ultrasound (58%) or AFP (≥20, 58%; ≥50, 47%) alone (220). Another study in patients with chronic viral hepatitis, reported that the use of AFP (>10 ng/mL) and ultrasonography resulted in higher sensitivity (100%) than either ultrasound (87%) or AFP (75%) alone (250).

In the single prospective randomized controlled trial of HCC screening 18,816 patients with chronic hepatitis B in Shanghai, China were randomized to undergo screening with both AFP and ultrasound on a 6-monthly basis, or no screening at all (251). Over an approximately 5-year period, patients were offered screening five to ten times; 58% of screening opportunities were accepted. In the screening group, the number of HCC lesions identified and resected was 86 and 40 (47%) versus 67 and 5 (8%) in the control group. In patients with HCC, the 5-year survival rate was 46% in the screening group versus zero in the control group. In this study, bi annual screening reduced HCC mortality by 37%.

Computed tomography (CT)

The classical appearance of a HCC on a dynamic CT scan of the liver is that of early arterial phase enhancement, followed by rapid washout on a later phase. A pseudocapsule if present generally shows delayed enhancement. Small well-differentiated lesions may be

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more difficult to detect because of a tendency to have similar attenuation than the surrounding liver tissue. This may be partially overcome by triple-phase CT scan where images are taken in the arterial, portal venous, and the delayed or equilibrium phases. In this type of imaging, the portal venous and delayed phase sequences are useful in identifying small, well-differentiated lesions (Fig. 4.39).

Table 4.2. Performance Characteristics of α-Fetoprotein for Hepatocellular Carcinoma Screening

Study	Number of patients	Population	Study design	Screening frequency	Mean follow-up (range)	HCC	AFP cutoff	Sensitivity (%)	Specificity (%)
Gambarin-Gelwan (220)	106	Cirrhotics	Retrospective	6 m	NA	19	≥20	58	91
							≥50	47	96
Nguyen (221)	312	HCV cirrhotics	Retrospective	NA	NA	163	>20	63	80
							>50	51	89
							>100	41	97
							>200	32	100
Peng (222)	205	Chronic hepatitis C	Retrospective	NA	NA	205	>20	65	87
Cedrone (223)	350	Chronic viral hepatitis	Retrospective	NA	NA	74	>20	55	88
Trevisani (224)	340	Chronic viral hepatitis	Retrospective	NA	NA	170	>20	60	91
							>100	31	99
							>200	22	99
							>400	17	99

HCV, hepatitis C virus.

Table 4.3. Performance Characteristics of α-Fetoprotein for Hepatocellular Carcinoma Screening Based on Prospective Studies

Study	Number of patients	Population	Study design	Screening frequency	Mean follow-up (range)	HCC	AFP cutoff	Sensitivity (%)	Specificity (%)

Sherman (225)	1,069	HBsAg +ve	Prospective	6 m	26 m (6–60 m)	11	>20	64	91
Oka (227)	260	Cirrhotics	Prospective	2 m	30 m	55	≥20	39	76
							≥100	13	97
Chalasani (228)	285	Cirrhotics	Prospective	6 m	15 m (6–42 m)	27	>20	63	87
							>100	41	97
							>200	27	100
Tong (231)	602	Chronic viral hepatitis	Prospective	6 m	7 y	31	≥21	41	94
Bolondi (229)	313	Cirrhotics	Prospective	6 m	56 m (6–100 m)	61	>20	41	82
Chen JG (226)	3,712	HBsAg +ve	Prospective	6 m	62 m	257	>20	55	87
Pateron (230)	118	Cirrhotics	Prospective	6 m	36 m (4–48 m)	14	>15	50	86
							>100	21	93

Table 4.4. Performance Characteristics of Ultrasound, Computed Tomography, Magnetic Resonance Imaging, and Postiron Emission Tomography for Hepatocellular Carcinoma Detection: Studies Comparing Imaging with Pathological Examination of Explanted Livers after Orthotopic Liver Transplantation or Partial Hepatectomy

				Ultrasound		CT		MRI	
Study	Patients	Population	Patients with HCC	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Rode (235)	43	Cirrhotics	13	46%	95%	54%	93%	77%	57%
Teefey (236)	25	Cirrhotics	9	89%	71%–75%	56%–67%	69%–75%	50%–56%	63%–81%
Gamberin-Gelwan (220)	106	Cirrhotics	19	58%	94%	53%	94%	NA	NA
Shapiro (237)	21	Cirrhotics with HCC	21	67%	100%	57%	100%	NA	NA
Kim (238)	52	Cirrhotics	18	38%	92%	NA	NA	NA	NA
Dodd (239)	200	Cirrhotics	34	50%	98%	NA	NA	NA	NA
Miller (240)	200	Cirrhotics	14[a]	NA	NA	68%	81%	NA	NA
Peterson (241)	430	Cirrhotics	59	NA	NA			NA	NA
Taourel (242)	35	Cirrhotics	9	NA	NA	89%[b]	88%	NA	NA
Yao (243)	70	Cirrhotics with HCC	45	62%[c]	NA	82%	NA	89%	NA
Bennett	200	Cirrhotics	27	30%	96%	NA	NA	NA	NA

(244)									
Llovet (245)	55	Cirrhotics	29	NA	NA	47%[d]	NA	100%[e] for all lesions (84%)[d]	95%
Takayasu (246)	100	HCC	100[f]	84%	NA	84% (93%)[b]	NA	NA	NA

[a]Includes one cholagniocarcinoma.
[b]CT with injection of iodized oil.
[c]included 11 additional patients with incidental HCC >2 cm found on explant.
[d]For lesions 11 to 20 mm
[e]MRI with angiography.
[f]Lesions <3 cm.
HCC, hepatocellular carcinoma.

Table 4.5. Performance Characteristics of Ultrasound for Hepatocellular Carcinoma Screening Based on Prospective Studies

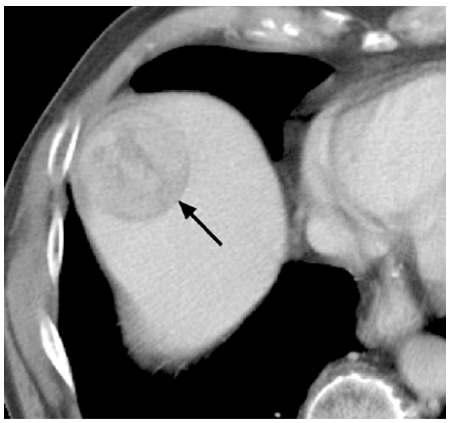
Study	Number of patients	Population	Study design	Screening frequency	Mean follow-up (range)	HCC	Sensitivity (%)	Specificity (%)
Pateron (230)	118	Cirrhotics	Prospective	6 m	36 m (4–48 m)	14	78	93
Sherman (225)	538	HBsAg +ve	Prospective	6 m	26 m (6–60 m)	11	79	94
Chen THH (247)	4,843	HBsAg +ve, HCV Ab +ve, ↑ ALT, AST, AFP, or family with a history of HCC	Prospective	3–12 m	7 y	93	78–100	98
Tong (231)	602	Chronic viral hepatitis	Prospective	6 m	7 y	31	100	98
Larcos (248)	232	Cirrhosis	Prospective	6–12 m	8 y	6	100	92
Chalasani (228)	285	Cirrhotics	Prospective	6 m	15 m (6–42 m)	27	59	93

HCC, hepetocellular carcinoma; HCV, hepatitis C virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, (α)-fetoprotein.

Proper timing of these sequences is crucial for the accurate characterization of the lesions, particularly for hypervascular lesions. HCC lesions typically demonstrate a hypervascular appearance with enhancement in the arterial phase, and rapid washout in the portal venous and delayed phases. Dysplastic nodules and regenerative nodules do not display this enhancement and usually appear similar or less attenuated than normal liver tissue. However, when the HCC is small, other benign lesions such as small hemangiomas, dysplastic nodules, and arterial to portal shunts can show homogenous arterial phase enhancement and mimick small HCC (252).

CT scan is often used to further evaluate lesions seen on ultrasonography. In studies comparing preoperative CT scan with the pathology of explanted livers (not screening studies), the sensitivity of CT scan has ranged from 47% to 89% with specificity ranging from 69% to 100%. The performance characteristics may have been influenced by the average tumor size in these studies.

Some studies have investigated the use of CT scanning for HCC screening. Although CT scan is useful for screening small groups of patients at high-risk for HCC, and for further evaluation of lesions seen on ultrasonography, it is an impractical modality for mass screening, particularly in less developed countries where it is cost-prohibitive. Also, repeated CT scans, every 6 to 12 months as part of a screening protocol, results in significant radiation exposure.



• **Figure 4.39** Hemochromatosis and hepatocellular carcinoma. Portal venous phase contrast-enhanced computed tomography demonstrates tumor (*arrow*) within the dome of the liver.

Chalasani et al. retrospectively reviewed 285 patients with cirrhosis who underwent initial screening for HCC with AFP, ultrasound, and CT scan testing followed by semiannual AFP and ultrasound imaging in patients who were considered eligible for liver transplantation. In this study, CT scan had a higher sensitivity (88%) than both AFP greater than 20 ng/mL (62%) and ultrasound (59%). However, other studies have not shown a benefit in tumor detection with the CT scan. In a study of patients undergoing triple-phase CT scanning, while awaiting liver transplantation, only 59% of HCC lesions were identified prior to transplant (241). In a retrospective analysis of 106 patients who underwent liver transplantation, the addition of CT scan to AFP and ultrasound did not increase the sensitivity of tumor detection (220).

Magnetic resonance imaging

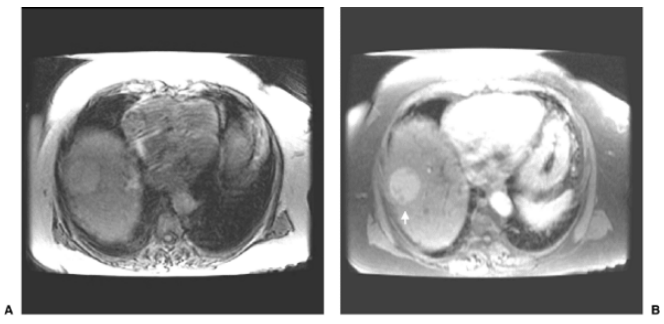
The appearance of HCC on MRI varies according to the characteristics of the lesion. Well-differentiated tumors generally appear hyperintense on T1-weighted images and isointense on T2-weighted images. Gadolinium-enhanced dynamic MRI allows the visualization of the liver in arterial, portal venous, and delayed phases (Fig. 4.40). Typical HCC appearance of arterial phase enhancement and portal venous phase washout allows the differentiation of these lesions from the less vascular regenerative and dysplastic nodules. MRI, however, like CT scan, does not perform well in the detection of small lesions.

In studies comparing preoperative MRI studies with the pathology of liver explants, the reported sensitivity and specificity rates have ranged from 50% to 100% and 57% to 95%, respectively. These results are similar to what has been reported for CT scan. MRIs are helpful in evaluating lesions that may represent HCC and has the advantage of not having the radiation exposure of CT imaging, but are considered too expensive for mass screening purposes.

Screening frequency

Screening for HCC with imaging is recommended at 6- to 12-month intervals (253). There have been no prospective comparisons of different screening schedules. The 6-month interval has evolved from studies that suggest the doubling time for HCC is approximately 3 months. In a study by Sheu et al. the growth rates of 31 asymptomatic HCC detected by ultrasound as part of a prospective screening program

were followed (254). The mean doubling time was 117 days (range 29 to 198 days, geometric mean 110 days). The only randomized trial of screening versus no screening showing a benefit of screening used 6-month intervals (251), a commonly applied approach in the United States. However, a randomized trial of 6- versus 12-month intervals showed no survival benefit for 6 months (255). These data are limited and do not support the widespread use of a longer screening interval.



• **Figure 4.40** Hepatocellular carcinoma. A 4 cm lesion in hepatic segment VIII seen before **(A)** and after **(B)** administration of gadolinium contrast on T1- weighted magnetic resonance images.

Outcome studies

Randomized, controlled studies comparing survival in patients undergoing HCC screening with controls who are not screened, are limited. Studies have looked at surrogates for survival including tumor size, tumor stage, and resectability in patients who have undergone screening. Several studies have demonstrated that HCC screening programs result in the detection of tumors that are smaller, and at an earlier stage making them more amenable to curative surgery (255,256,257,258). An improvement in patient survival based on screening has yet to be conclusively demonstrated, but has been suggested in retrospective studies (224,255,259). Furthermore, all these studies may be subject to lead-time bias.

Three large published screening trials will be discussed here. Two US-based studies screened HBsAg-positive patients. In the study by

Sherman et al. 1,069 hepatitis B carriers underwent AFP or AFP with ultrasonography every 6 months (225). Over a 5-year period, 14 hepatocellular carcinomas were identified. In seven of these patients, curative resection was not possible at the time of tumor detection because of tumor stage, and one additional patient refused surgery. Six patients underwent surgery with a curative intent; HCC recurred in one patient at 4 months. HCC had not recurred in the remaining five patients at follow-up periods ranging from 6 to 38 months, which is not a long enough duration to exclude tumor recurrence. A study screening 1,487 Alaskan natives with chronic hepatitis B were screened with AFP measurements every 6 months for a 16-year period (260). Patients with an elevated AFP underwent ultrasound examinations. HCC was detected in 32 patients; tumors less than 6 cm were found in 23 patients, of whom 22 underwent resection. The 5- and 10-year survival rates for patients diagnosed with HCC during this trial were 42% and 30%. A historical control group was selected of HBsAg positive patients diagnosed with HCC prior to the study; none of those 12 patients survived beyond 2 years after HCC diagnosis. Finally in a study of 1,125 patients with chronic viral hepatitis who underwent AFP and ultrasound examinations every 3 months, HCC was diagnosed in 67 patients (250). Median survival in the 24 patients who underwent tumor resection was 26 months, compared to 6 months in the 43 patients with unresectable tumors.

Unresolved questions regarding hepatocellular carcinoma screening

Large, prospective, randomized controlled trials are needed to definitively establish the optimal HCC screening modality and frequency that would result in a survival benefit. Such studies would be challenging to conduct, given the large number of patients required and the ethical dilemmas related to conducting such a study.

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Lindor KD, Bru C, Jorgensen RA, et al. The role of ultrasonography and automatic-needle biopsy in outpatient percutaneous liver biopsy. *Hepatology* 1996;23:1079–1083.

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Chapter 5

Interventional Radiology Procedures for Diagnosis and Treatment of Hepatobiliary Disease

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Douglas M. Coldwell

Key Concepts

- Advances in noninvasive imaging and endoscopy continue to modify the indications for interventional procedures, some of which, such as transabdominal splenoportography, have been altogether abandoned. Other procedures such as hepatic arteriography and transhepatic percutaneous cholangiography are infrequently required for diagnosis.
- Catheter-based assessment of the portal and hepatic venous systems remains important for a number of conditions. There are several safe ways of studying the portal venous system.
- Angioplasty and stenting have become important adjuncts for treating arterial, venous, and biliary obstructions.
- Liver biopsy can be performed transabdominally with a high degree of safety and excellent diagnostic yield. Transjugular biopsy is reserved for patients with diffuse liver disease and contraindication to transabdominal biopsy.
- Tumor ablative therapies include transcatheter and percutaneous methods. There are recent data to support the use of transarterial chemoembolization of hepatic malignancies when curative resection is not considered.
- Percutaneous drainage has become a cornerstone in treatment of liver abscesses.

Ongoing advances in noninvasive imaging have rendered many diagnostic interventional methods of the last decade obsolete. Computed tomography (CT), magnetic resonance imaging (MRI), ultrasound, and radionuclide imaging often provide sufficient diagnostic information for medical management and surgical planning. Endoscopic techniques have mostly replaced percutaneous transhepatic cholangiography (PTHC) and percutaneous biliary intervention. Diagnostic angiography and PTHC are now reserved for those cases in which less invasive diagnostic evaluation proves inconclusive.

What remains under the heading of interventional radiology procedures, therefore, includes an aray of diagnostic procedures that are used when noninvasive imaging is just not adequate, alongside a growing number of interventional therapies for treating both benign and malignant conditions. Many percutaneous therapeutic interventional procedures have been developed to ablate hepatic malignancies, including transarterial chemoembolization (TACE), various percutaneous probe-directed ablative therapies, and most recently, selective internal radiation therapy (SIRT) performed by

transarterial delivery of radioactive particles. Although many of these ablative procedures are considered safer than open surgeries, validation of the results in well-controlled comparative studies has been lacking, except for TACE in which recent randomized trials confirm the usefulness of this technique.

This chapter addresses a broad spectrum of percutaneous diagnostic and therapeutic procedures, attempting to temper unfounded enthusiasm while noting those areas where invasive techniques offer safe and effective medical care for patients with hepatobiliary disease.

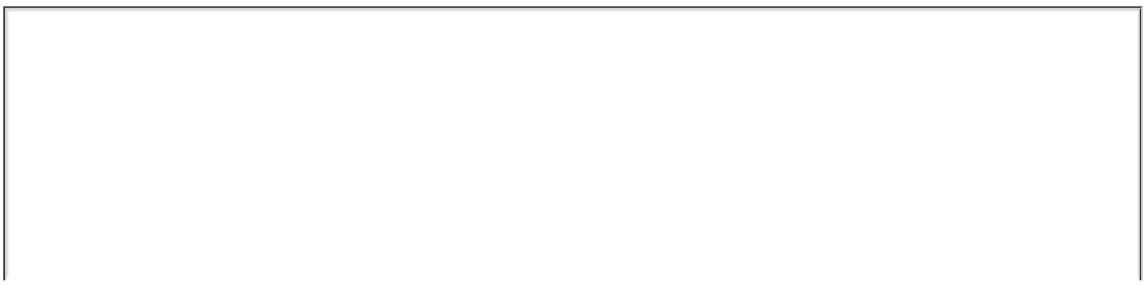
Angiography

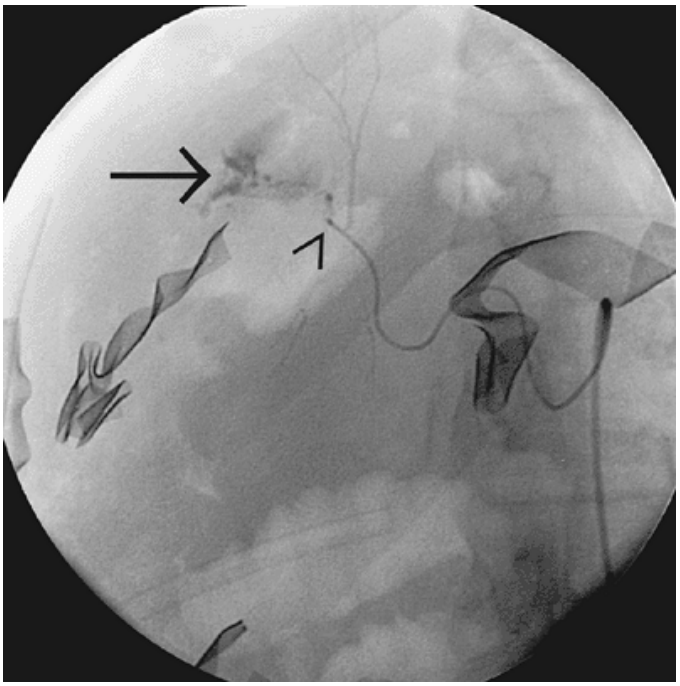
These days the term *angiography* requires some clarification. Once only the domain of percutaneous catheterization, angiography (visualization of arteries and veins) can now be done by a number of imaging methods without the need for direct vascular puncture. Several methods do not even require the administration of any type of contrast agent. Examples include color-flow sonography and magnetic resonance angiography (MRA) using imaging techniques based on the physical properties of flowing blood to distinguish blood vessels from static tissues. Not only can these two techniques image blood vessels without using contrast agents and percutaneous puncture but they can also avoid ionizing radiation and virtually all the risks that accompany catheter-based angiography. With the addition of intravenous contrast administration, both CT scan and MRA are capable of rendering arteriograms and venograms that often rival conventional angiograms obtained with catheterization techniques. Furthermore, noninvasive angiography with sonography, CT scan, and MRA techniques are far less expensive than catheter-based angiography. These noninvasive methods also demonstrate anatomy beyond the blood vessels and can show changes in the soft tissues and bones that may not be seen with conventional angiography.

If these noninvasive methods produce angiograms that are consistently comparable to conventional angiograms in spatial and contrast resolution there will be no reason to insert a catheter for vascular diagnosis ever again. Noninvasive angiograms obtained with ultrasound, CT scan, and MRA, however, are not always adequate for arriving at a diagnosis or may not be sufficient to guide further therapy. Therefore, catheter-based angiography is still required in a number of clinical situations.

Hepatic Arteriography

The principles and practice of catheter-based hepatic arteriography have improved during the last decade. Catheter size, measured by outer diameter of the catheter, has been reduced to 4 to 5 Fr (1 Fr = 0.33 mm). This translates to a smaller arterial puncture at the groin and a theoretic reduction in puncture site complications. Introduction of the Glide Wire (Boston Scientific, Natick, MA) in clinical practice has facilitated visceral catheterization as well. Our ability to reach distant branch vessels has been further enhanced by the introduction of "microcatheters" that are inserted through a standard 5-Fr angiographic catheter and directed toward the target artery with "microwires." It is now routinely possible to enter vessels in the liver that are fourth- and fifth-order branches, typically 0.5 to 1.0 mm in diameter, regardless of the degree of vascular tortuosity (Fig. 5.1).





▪ **Figure 5.1** Digital angiogram of a superselective hepatic artery catheterization. Contrast extravasation (*arrow*) is seen at the site of the traumatic liver laceration. The microcatheter tip (*arrowhead*) has been placed in a fourth-order right hepatic arterial branch.

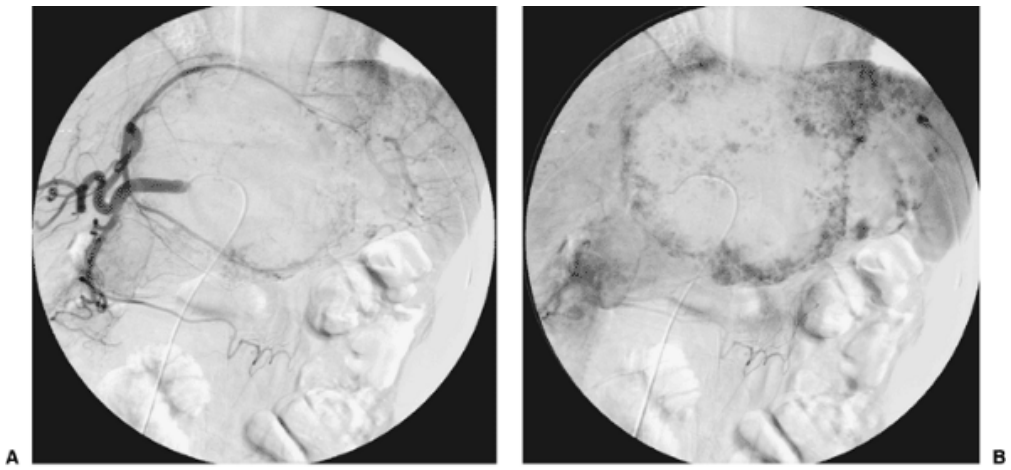
Fluoroscopic and digital angiography equipment have also improved. State-of-the-art systems have been available for most of the last decade and afford up to four times the resolution of earlier digital imaging systems. Additional developments permit the angiographer to freeze an angiographic image on top of a live fluoro image, thereby providing a "roadmap" on which difficult catheterizations can be performed.

A number of other advances make hepatic arteriography safer than ever. These include the use of low osmolar contrast agents that reduce the discomfort and idiosyncratic reactions caused by iodinated contrast injection; the widespread use of hemodynamic monitoring, permitting the safe use of conscious sedation; and a variety of recently introduced puncture closure devices. These puncture closure devices use absorbable sutures, bovine collagen, thrombin, or a combination of these agents. They have been approved for use in patients who are anticoagulated and may provide a margin of safety when arterial catheterization is performed in a patient with known coagulopathy (i.e., prolonged prothrombin time and thrombocytopenia), a condition often seen in patients with liver disease.

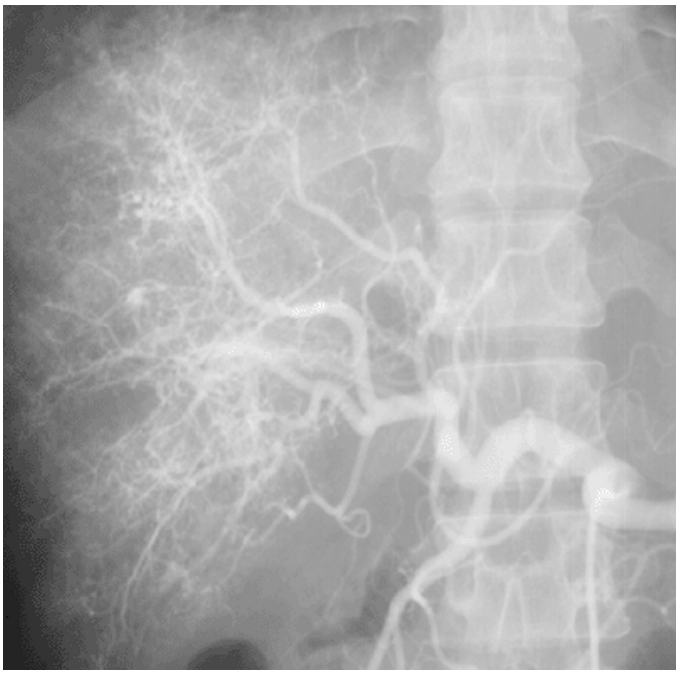
Although hepatic arteriography is safer and more diagnostic than ever before, the indications for arteriography of the liver have declined as noninvasive imaging methods have improved. For example, it is rarely necessary to demonstrate the arteriographic "starry night" early phase and vascular lakes appearance on delayed

images that are characteristic of a cavernous hemangioma in the liver (Fig. 5.2A,B) because MRI, CT, ultrasound, and radionuclear imaging can make the diagnosis in most cases. The same is true for most hepatocellular carcinomas (Fig. 5.3) and metastases (Fig. 5.4A,B) in which noninvasive imaging may be followed by percutaneous biopsy if indicated, avoiding the need for arteriography. Catheterization of the mesenteric arteries for CT arterial portography is now of dubious value because noninvasive

imaging methods such as multiphase CT scanning show similar sensitivity for detecting small (<1 cm diameter) liver lesions without arterial catheterization (1,2,3). The reality is that hepatic angiography is only occasionally used as a diagnostic test. More often, angiography is used to guide percutaneous procedures such as embolization, angioplasty, and stenting or to create a vascular map for the surgeon who contemplates shunt surgery or liver transplantation when noninvasive imaging is not sufficient.



▪ **Figure 5.2** Early-phase and late-phase arteriogram of a cavernous hemangioma of the liver. **A:** Displacement of both branches of the left hepatic artery by this extremely large cavernous hemangioma. This type of vascular displacement is not typical of hemangiomas until they become massive. Early filling of the hemangioma is noted by a faint "starry night" appearance at the end of the small arterial branches. **B:** Characteristic contrast lakes are seen at the periphery of the cavernous hemangioma as the vascular spaces within the hemangioma fill during angiography.



▪ **Figure 5.3** Hepatocellular carcinomas. There is diffuse neovascularity, seen as a cobweb pattern, throughout the right lobe of the liver in this patient with multifocal hepatocellular carcinomas.

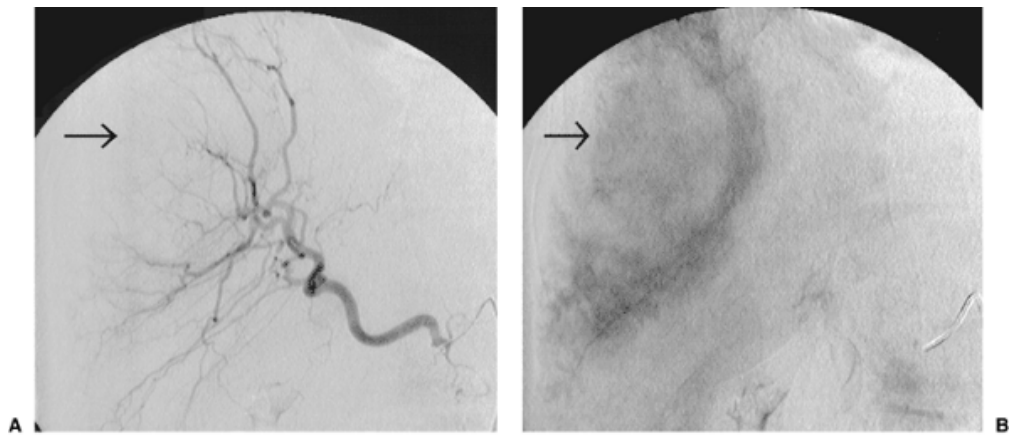
Portal and Hepatic Venography

Although arteriography is on the decline, venography of the portal venous system and hepatic veins remains an important procedure for diagnosis and guidance of therapy.

Portal venography

Venographic imaging of the portal vein is often required in the preoperative assessment of portal hypertension before shunt surgery and, occasionally, before liver transplantation. It is also an important component of the transjugular intrahepatic portosystemic shunt (TIPS) procedure and used on occasion during the assessment of Budd-Chiari syndrome (BCS). There are four angiographic methods that can be used to image the portal vein and its tributaries which are as follows:

1. Arteriographic portography
2. Transvenous portography



▪ **Figure 5.4** Early-phase and late-phase hepatic arteriogram of metastatic disease to the liver. **A:** There is splaying of the right hepatic arterial branches by a large colorectal metastasis (*arrow*). **B:** Late arterial phase angiogram shows the hypovascular defect produced by the metastasis (*arrow*) with some hypervascularity in the adjacent liver that may represent compression of normal liver tissue by the metastasis.

3. Transabdominal–transhepatic portography
4. Transabdominal–transsplenic portography

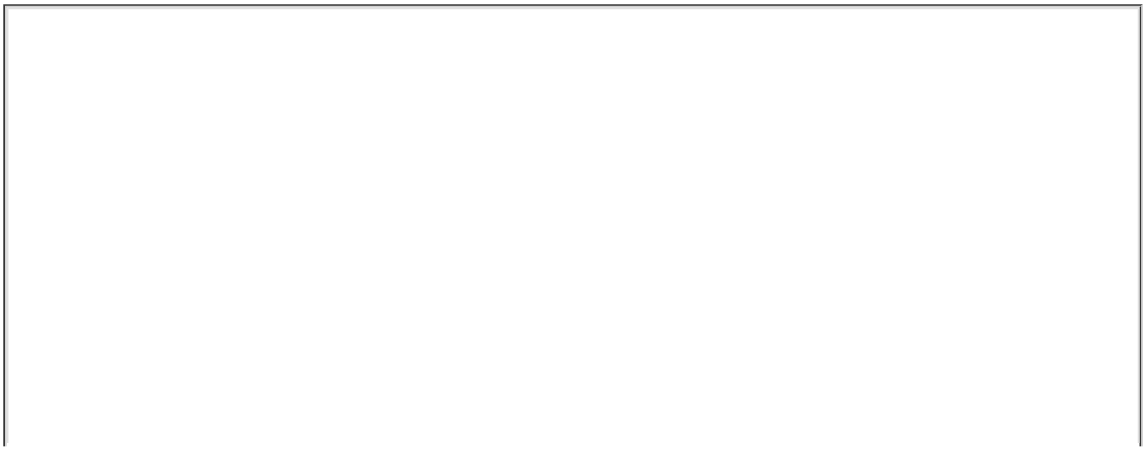
Arteriographic portography implies arterial catheterization with selective contrast injection into the splenic artery, superior mesenteric artery, or inferior mesenteric artery. After contrast passes through the visceral capillaries, it enters the portal

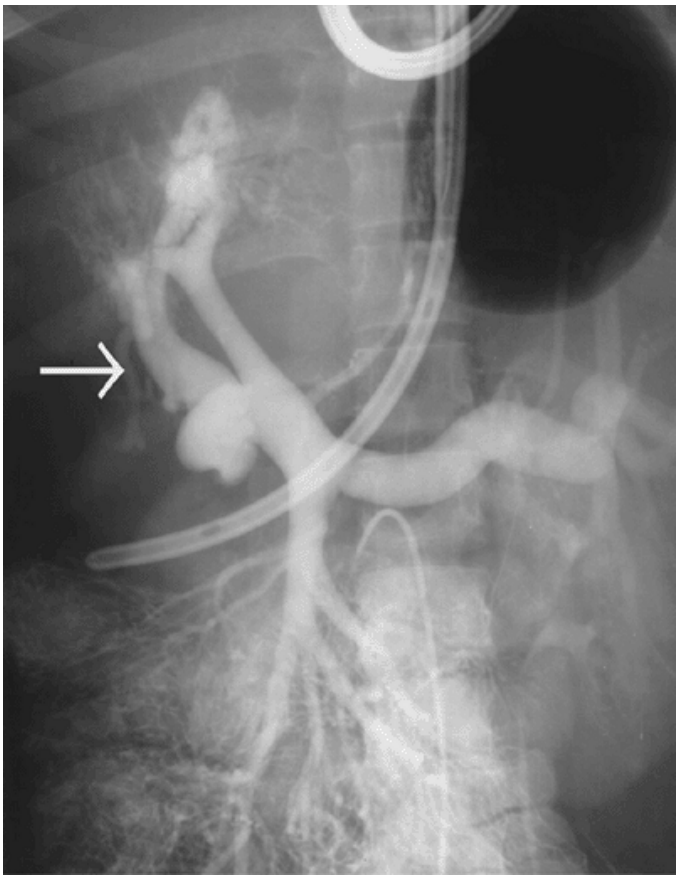
venous tributaries and then the portal vein itself. "Late" filming after arterial injection of contrast will therefore demonstrate the entire portal venous system in most patients. This method is the best way of assessing dynamic flow patterns in the portal venous system because it images all portal venous branches, collaterals, and varices as they fill with contrast. This is important when assessing flow dynamics of variceal filling before surgery. For example, dynamic portal venous flow patterns often guide the surgeon during portocaval shunting, distal splenorenal shunting, and devascularizing procedures of gastroesophageal varices by delineating the relative importance of flow into the left gastric vein (coronary vein), splenic vein, and umbilical vein (recanalized paraumbilical venous collateral system) (Fig. 5.5).

Limitations of arteriographic portography include dilution of contrast by nonopacified blood before reaching the portal system, which often limits visualization. This is a particular problem when contrast is injected into the splenic artery of patients with hypersplenism; in such cases contrast does not pass swiftly through the enlarged spleen into the portal venous system. Portal vein filling may be delayed for more than 20 or 25 seconds in patients with portal hypertension, and it is frequently not possible for a patient to hold his breath for this duration during the imaging sequence. When a patient breathes during digital subtraction angiography, the motion of the liver and bowel may obscure faint portal venous filling. Finally, in some patients with high portal venous pressure, hepatofugal flow may prevent portal vein opacification altogether.

Transvenous portography is performed by systemic venous puncture, advancing a catheter through the hepatic vein until it becomes wedged in one of the small hepatic vein branches. Iodinated liquid contrast or carbon dioxide (CO_2) gas is then injected into the catheter, which passes back from the small hepatic vein into the hepatic sinusoids and finally retrograde into the portal vein (4). CO_2 gas is now preferred over iodinated liquid contrast agents because it affords more reliable and complete filling of the portal vein. When attempting transvenous CO_2 portography, digital subtraction angiographic equipment is needed because CO_2 gas is difficult to distinguish from adjacent soft tissues without the subtraction technique (Fig. 5.6). Although widely adopted for portal vein visualization during transhepatic portosystemic shunt formation for the treatment of portal hypertension, this method is limited by several factors that are typically not known before attempting the procedure. First, the degree of sinusoidal destruction due to cirrhosis or inflammation may impede an adequate volume of CO_2 gas from crossing through the sinusoids and entering the portal system for adequate visualization of the portal vein. Also, in some patients it is difficult to wedge the catheter with sufficient force to prevent the reflux of CO_2 into the hepatic vein that has been

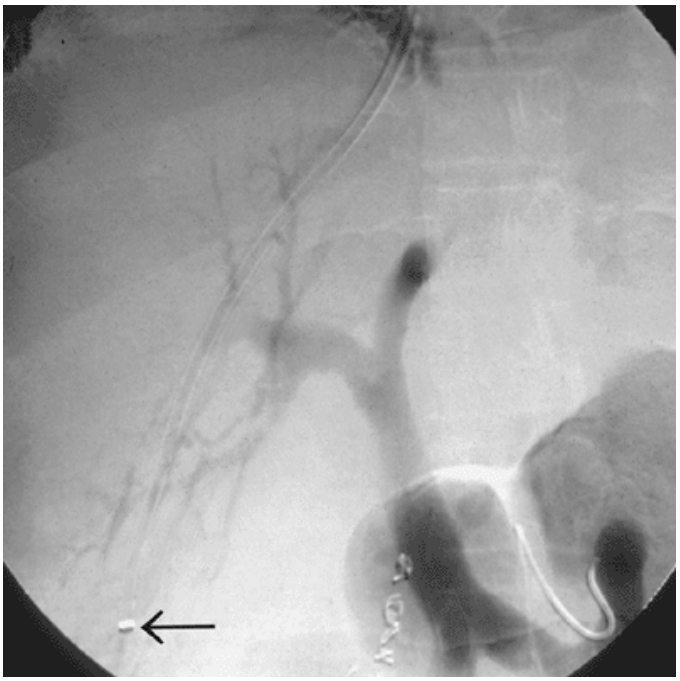
catheterized. Finally, this technique only rarely depicts the portal system proximal to the main portal vein because CO_2 flash fills the main portal vein and its intrahepatic branches for 1 to 2 seconds before the portal circulation forces it back into the sinusoids.





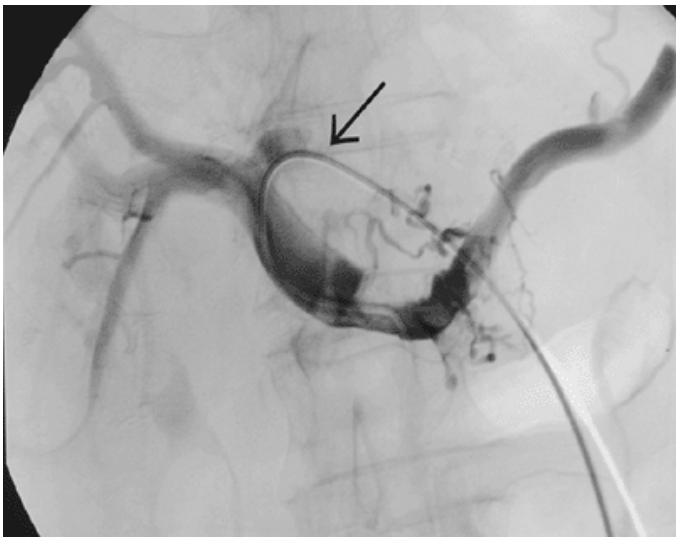
▪ **Figure 5.5** Schistosomiasis with sinusoidal portal hypertension. This is the late phase (portal venous phase) of a superior mesenteric artery angiogram, in which expected filling of the superior mesenteric vein, occurs and the relatively small main portal vein, as well as some hepatofugal filling of the splenic vein. Note the recanalized paraumbilical vein (*arrow*) that arises from the left portal vein and travels in the falciform ligament toward the umbilicus.

There have been several case reports of fatality after transvenous portography when forceful injection of liquid contrast agents or CO_2 gas has ruptured the liver capsule rather than having passed through the sinusoids (5,6). Breach of the liver capsule can be immediately recognized on the venographic images obtained during the attempted transvenous portogram. Transcatheter embolization of the tear should be performed immediately using gelfoam pledgets and/or occlusive coils in an attempt to halt bleeding into the peritoneal cavity. Even when these measures are taken, it is possible that a capsular tear will result in excessive intraperitoneal hemorrhage. Therefore, to avoid capsular tear, transvenous portography should be attempted after making all reasonable efforts to wedge the catheter in an hepatic vein branch located within the central substance of the liver far from the liver capsule. This can be facilitated by reviewing the CT, MRI, or ultrasound studies that have invariably been done before performing transvenous portography, noting whether there is excessive atrophy of the right lobe of the liver, which would preclude safe injection into the right or middle hepatic veins (MHVs).



▪ **Figure 5.6** Wedged transvenous carbon dioxide (CO_2) portogram. A vascular sheath is introduced through the right internal jugular vein, with its tip (*arrow*) wedged in a small venous tributary to the right hepatic vein. CO_2 is passed through the sinusoids and refluxed into the portal venous system, with excellent visualization of the right, left, and main portal veins on the digital subtraction angiogram.

Transabdominal–transhepatic portography was first described in 1974 by Lunderquist and Vang (7). Although it is infrequently used in contemporary practice, we have found this to be a safe and relatively easy way of entering and imaging the portal venous system. The technique requires ultrasound guidance for transabdominal (transhepatic) puncture into the left or right portal vein. The left portal vein is preferred because it is closer to the skin than the right portal vein and compression can be applied over the hepatic puncture site in most patients when the catheter is removed. Once the puncture needle enters the right or left intrahepatic portal vein, a guide wire is introduced and the needle is replaced with a small (5 Fr) hemostatic sheath. An angiographic catheter can be placed into the portal system through this sheath and used to image the entire portal venous system. Pressure measurements can also be obtained directly. Another advantage is that selective contrast injections can be made into the portal vein branches, and sequential imaging can then demonstrate the flow dynamics with a much better depiction of the portal vein anatomy than transarterial portography.



▪ **Figure 5.7** Transabdominal-transhepatic portal venogram. The catheter is placed into the left portal vein branch with sonographic guidance (*arrow*), and it passes retrograde into the main portal vein. The tip of this catheter is at the confluence of the splenic vein and main portal vein. Partially occlusive thrombus, adjacent to the catheter tip, was treated using thrombolytic therapy.

We have used this method when approach the portal system while providing transcatheter therapies such as thrombolysis of portal vein clot (Fig. 5.7) or embolic occlusion of a vascular shunt that involves the portal venous system. In fact when Lunderquist and Vang first described this technique, it was used to provide access for transcatheter embolization of the coronary vein.

Transabdominal–transsplenic portography is only of historical interest. This procedure has a complication list that includes significant hemorrhage (500 to 2,000 mL) in up to 2% of patients (8), with the need for splenectomy in some of these patients. Additionally, there is an increased incidence of splenic artery branch pseudoaneurysms because of laceration caused by the needle tip (9). Given the development of the three other methods for portal venous imaging, as well as the use of transhepatic portal vein catheterization for obtaining pressure measurements (and hepatic vein catheterization with free/wedge hepatic vein pressure measurements, as described in the following text), there is no longer a clinical need for this procedure.

Portal pressure measurements

As noted in preceding text, direct entry into the portal venous system is possible by a transabdominal–transhepatic approach using ultrasound-directed puncture into an intrahepatic portal vein branch. Although we have found that this approach to be technically straightforward and safe, it is possible to obtain information about portal venous pressure quite simply (if indirectly) by catheterization of one of the hepatic veins. The portal venous pressure is calculated by measuring the wedged hepatic vein pressure and the "free" hepatic vein pressure (pressure in the hepatic vein without wedging the catheter tip). Subtraction of the free hepatic vein pressure from the wedged pressure yields an indirect calculation of the portal vein pressure, called the *corrected sinusoidal pressure* (*CSP*). The CSP is normal (<5 mm Hg) when there is no portal hypertension and when there is portal hypertension due to presinusoidal portal venous occlusion (such as main portal vein thrombosis). The CSP is elevated in

sinusoidal and postsinusoidal causes of portal hypertension, with values between 6 and 10 mm seen in mild portal hypertension and between 11 and 15 mm Hg in moderate portal hypertension (10).

Other methods to measure portal venous pressure include direct entry into the portal vein by a transjugular intrahepatic puncture (the first step in creating a TIPS shunt). This is technically more challenging than the transabdominal–transhepatic approach and is usually reserved for portal vein pressure measurements only during the TIPS procedure. Finally, portal venous pressures were obtained for many years by transsplenic puncture and measurement of the splenic pulp pressure, but as noted in the preceding text, there is no longer any reason to perform this procedure because other methods are safer.

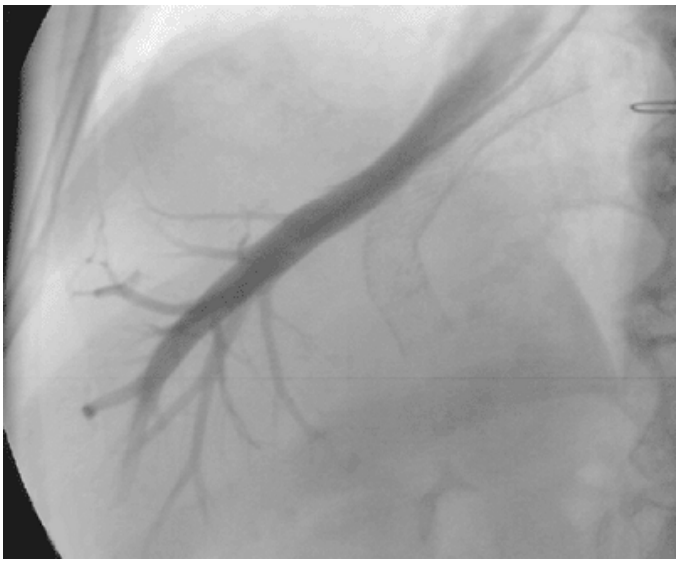
Hepatic venography

Hepatic venography remains an important procedure for the evaluation of BCS and for the assessment of certain diffuse hepatic processes such as veno-occlusive disease. It is also necessary during transjugular liver biopsy and TIPS, and for the occasional evaluation of vascular malformations of the liver.

This procedure is typically performed by puncturing the internal jugular vein because the hepatic veins are most easily catheterized by gaining entry into the inferior vena cava (IVC) from above. Nevertheless, some interventionalists prefer entry by a femoral vein puncture. In case of BCS, it may be necessary to perform hepatic venous catheterization using both the jugular and femoral approaches because IVC stenosis or occlusion due to a web or compressive effect on the IVC caused by hepatic hypertrophy may confound the interventionalist who attempts this procedure only using the jugular vein approach. As noted in the preceding section, the usefulness of hepatic venography may be augmented by wedged and free hepatic vein pressure measurements if portal hypertension is suspected.

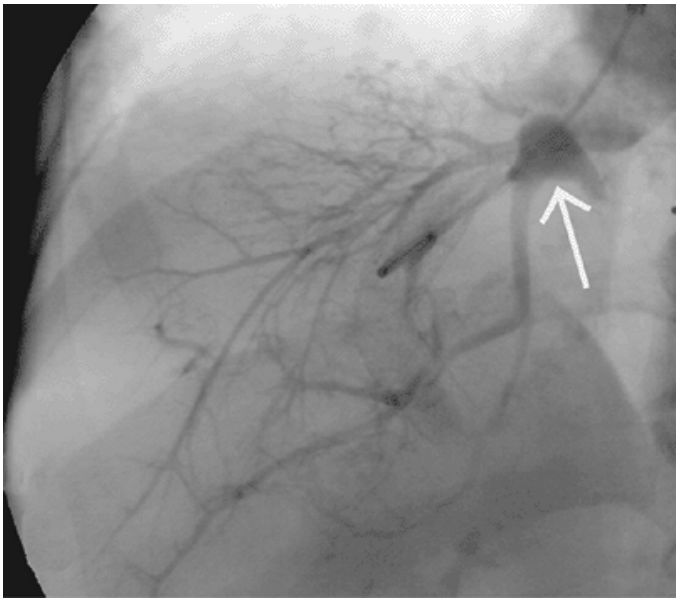
Venous anatomy of the liver can be studied by selective catheterization of each of the three hepatic veins. The right hepatic vein enters directly into the IVC (Fig. 5.8), whereas the MHV and left hepatic vein (LHV) form a common trunk just before entering the IVC (Fig. 5.9). Other hepatic veins include small caudate veins that typically enter directly into the IVC below

the level of the dominant hepatic venous drainage, as well as the inferior right hepatic vein (IRHV) that enters the IVC at the level of the caudate lobe (Fig. 5.10).

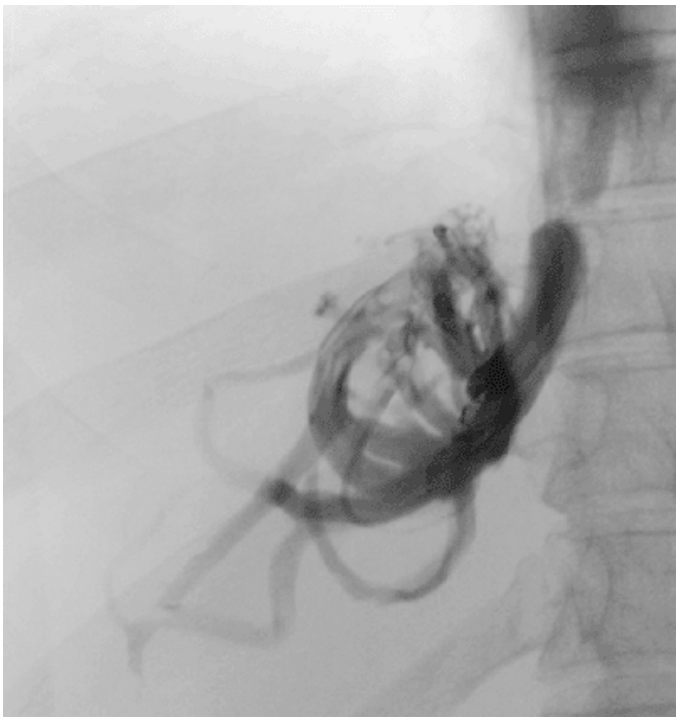


▪ **Figure 5.8** Right hepatic venogram. The catheter is introduced from the right internal jugular vein.

As mentioned in the preceding text, venographic findings in BCS include thrombosis or webs of the hepatic veins and stenosis of the IVC due to a web (Fig. 5.11A,B) or hypertrophy of the left lobe of the liver. Contrast, on being injected into diseased hepatic veins central to the occluding clot or webs, will often fill a rich network of intrahepatic venous collaterals that bridge the hepatic veins (Fig. 5.12A,B) and may collateralize to the portal and systemic venous systems.



▪ **Figure 5.9** Middle and left hepatic veins of the same patient as in Figure 5.8. The *arrow* indicates the confluence of these two venous branches. There is excellent filling of the middle hepatic vein branches and limited filling of the left hepatic vein branches.



▪ **Figure 5.10** Catheterization of the inferior right hepatic vein (IRHV) in a patient with an inferior vena caval web (see Fig. 5.11 for additional venograms of this patient).

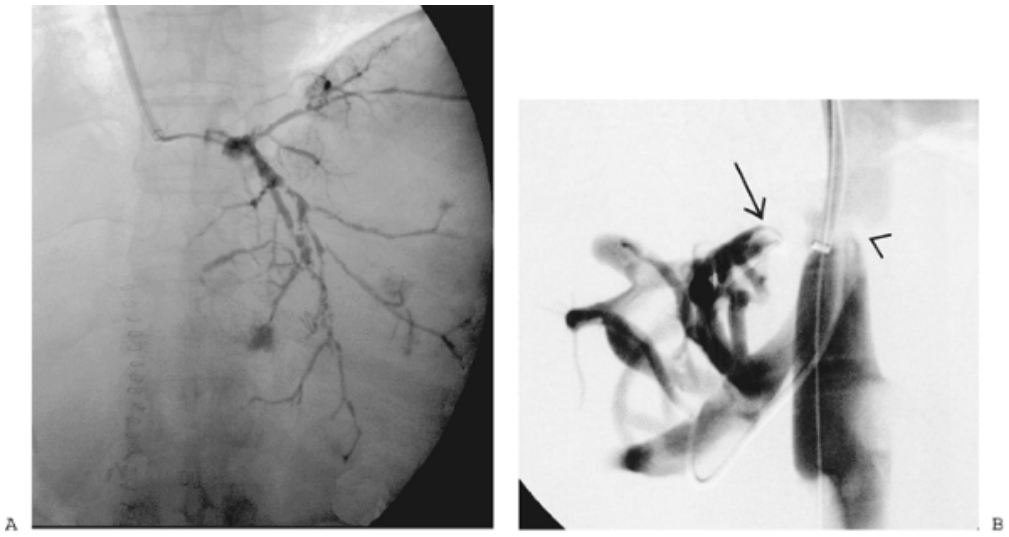
Percutaneous Transhepatic Cholangiography

Developed through the 1960s and 1970s, the current technique for PTHC was revolutionized by the Japanese, with the introduction of a long, thin, flexible needle named the *Chiba needle* in honor of the city in which it was invented (11,12,13). PTHC, although relatively safe, carries the risk of cholangitis, sepsis, and hemorrhage. It is therefore of little surprise that noninvasive tests and endoscopy have replaced PTHC in the diagnosis of most types of biliary disease.

Contemporary assessment of biliary disease usually begins with ultrasound to assess biliary dilatation/obstruction. Endoscopy can often reveal the etiology and location of the biliary obstruction and the endoscopist can often insert a catheter for biliary drainage. Subsequent imaging evaluation may include CT scanning (to look for stones, a pancreatic head mass, masses in the porta hepatis, or intrahepatic disease), scintigraphy (to assess biliary excretion), and magnetic resonance cholangiopancreatography (MRCP) (to evaluate the biliary anatomy).

The most frequent indications for PTHC include suspected biliary obstruction at or above the common hepatic duct, failure of endoscopic cannulation of the ampulla of Vater due to prior surgery or other anatomic factors, prior Roux-en-Y anastomosis, nondiagnostic MRCP, or when percutaneous access into the biliary

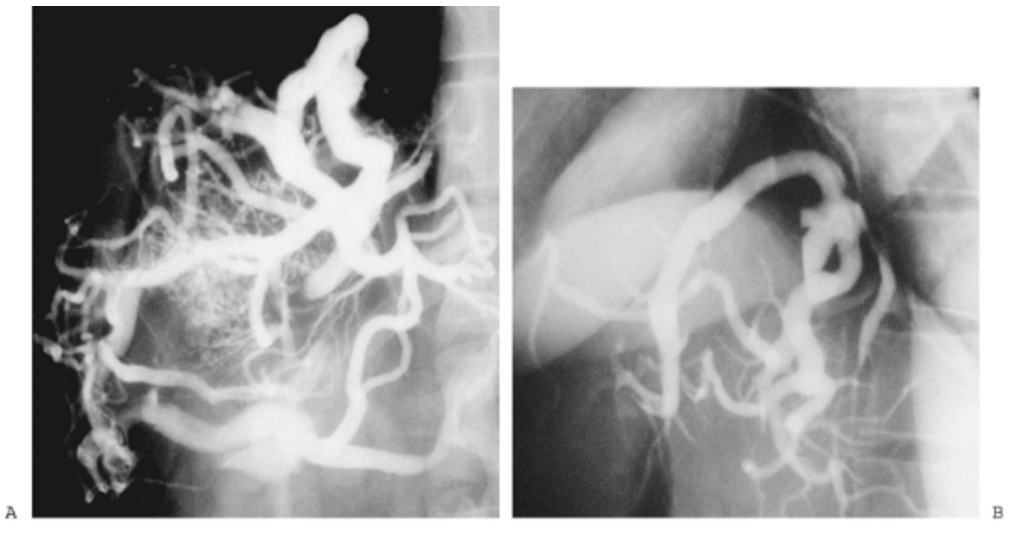
tree is needed for drainage, biopsy, stone manipulation, or other interventions.



▪ **Figure 5.11** Left hepatic venogram and inferior vena cavagram in a patient with Budd-Chiari syndrome. **A:** Multiple branches of the left hepatic vein undergo thrombosis and recanalization, giving a "scrawny" appearance to the veins that fill with contrast. **B:** A web in the inferior vena cava is noted (*arrowhead*), and the occluded right hepatic vein (*arrow*) fills through inferior right hepatic vein collaterals.

Patient preparation is essential for safe performance of PTHC. We always administer prophylactic antibiotics before performing PTHC and typically provide antimicrobial coverage for gram-positive cocci and gram-negative rods using ampicillin with tobramycin, gentamicin, or a second-generation cephalosporin. Additionally, all reasonable efforts should be made to correct any abnormal coagulation parameters before performing PTHC, including normalization of the prothrombin time and transfusion of platelets during the

procedure if necessary. Although ascites is a relative contraindication to PTHC, there are ways to avoid the complications of peritonitis and intraabdominal hemorrhage, such as drainage of the ascitic fluid, before PTHC.



▪ **Figure 5.12** Budd-Chiari syndrome—anteroposterior (AP) and lateral venograms. **A:** AP hepatic venogram shows the chaotic appearance of an engorged hepatic venous system. **B:** Lateral hepatic venogram shows that there is no identifiable outflow from the hepatic veins into the inferior vena cava.

PTHC may be approached from either the right or the left side of the liver. We prefer a left-side approach regardless of whether there is biliary dilatation. Access into the left intrahepatic bile duct avoids transpleural puncture, reduces respiratory motion of the liver during the procedure, and permits extrinsic compression of the puncture site if necessary. Furthermore, injection of contrast into the left biliary tree usually yields complete filling of the bile ducts in both hepatic lobes because iodinated contrast is denser than bile and flows down into the dependent right biliary system as the patient lies supine. When there is distal obstruction of the extrahepatic bile duct, the left bile duct is typically more dilated than the right and, therefore, more easily punctured. Radiation dose is always of concern when working from a left hepatic lobe puncture because there is a tendency to cross one's hands in front of the x-ray beam. But since entry into the biliary tree is facilitated from the left side using ultrasound guidance, we believe that the total radiation exposure of the operator's hands to the x-ray beam, as well as exposure from scattered radiation emanating from the patient, will be less than that sustained from a right-side puncture under fluoroscopy alone.

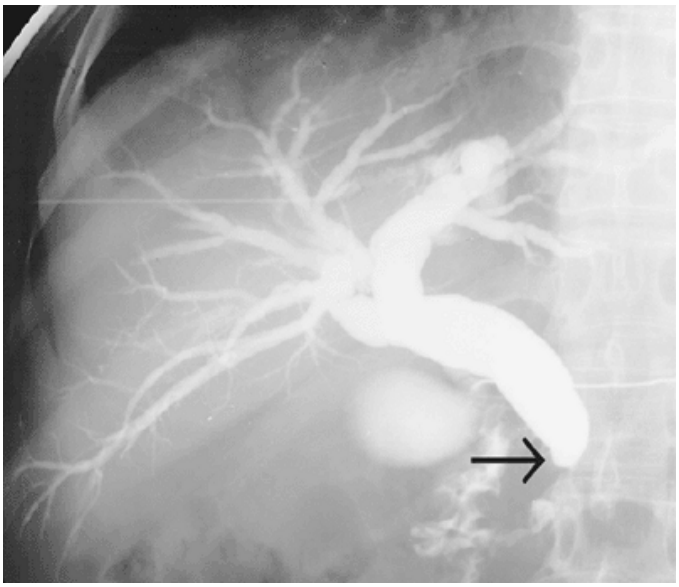
Ultrasound is used in virtually every PTHC that we perform because it guides puncture into the bile duct, often reducing the interventionalist's efforts to only one needle pass. Even when intrahepatic bile ducts are not dilated, ultrasound is used to identify the left portal vein that serves as a reliable marker for the portal triad (and therefore the left biliary ductal system). An acceptable site of entry into the biliary tree is especially important when biliary drainage is considered. The site of entry should not be too peripheral because the biliary radicals are small and close to the liver capsule where leakage of bile can cause peritonitis. Likewise, the puncture should not be too central because injury to the hepatic artery is more likely. Usually a second-, third-, or fourth-order biliary radical is preferred. But with a nondilated system, virtually any bile duct will suffice for diagnostic PTHC because there is no guarantee that additional needle passes will have any success in finding an optimal duct for opacification.

Excessive filling of the biliary tree with contrast may lead to sepsis and death if bile is colonized or infected. This may occur even when prophylactic antibiotics are given because the bacteria that are already in the bile cause sepsis by releasing endotoxin into the bloodstream. Whereas overfilling is dangerous, underfilling may render the study nondiagnostic. This is a dilemma for the interventionalist, who relies on his experience and judgment during PTHC. Once the system is filled with adequate contrast, imaging should be performed in multiple projections. Obstructions should be evaluated accurately to determine whether they represent stones, strictures, or tumors. The ease with which contrast flows past a stricture is particularly important when assessing a surgical anastomosis because there is typically some narrowing after surgical reanastomosis of the bile duct to the bowel that may not represent a functional obstruction. Manometry has been used to determine the significance of benign biliary strictures, as described in the following text. For malignant strictures, determination of the location of the obstruction is crucial because it may render a malignant obstruction amenable to surgical bypass (and even potential cure) or may convince the surgeon that curative surgery is fruitless and that nonoperative palliative measures are warranted.

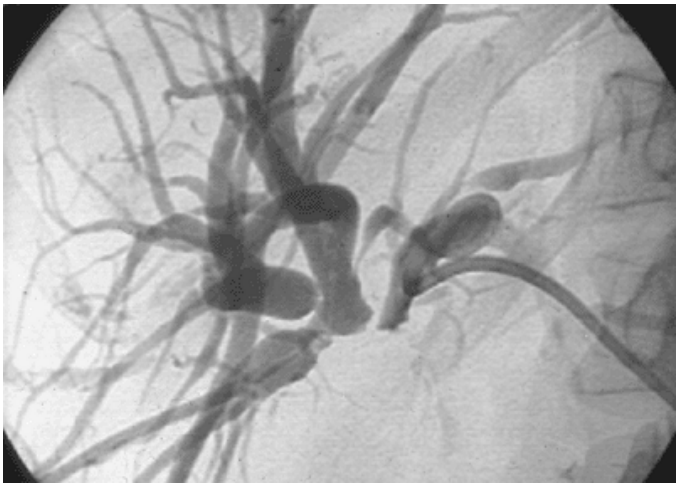
There have been many papers that describe the findings of stones, amorphous sludge, cholangitis, cholangiocarcinoma, benign strictures, and sclerosing cholangitis. Despite the degree of certainty with which some authors distinguish these conditions, it is

often not possible to distinguish sclerosing cholangitis from cholangiocarcinoma, or stones from sludge. Nevertheless, stones are typically identified as round or oval luminal filling defects that do not change in shape or size. Air bubbles that may have been introduced inadvertently during the procedure are usually round, mobile filling defects that can often be aspirated back through the needle. Obstruction of the distal common bile duct with an abrupt taper usually represents pancreatic carcinoma (Fig. 5.13). Obstruction of the

middle or high extrahepatic duct represents cholangiocarcinoma or extrinsic metastases (Fig. 5.14). When the intrahepatic ducts are occluded, the differential diagnosis includes cholangiocarcinoma, sclerosing cholangitis, and malignancy or lymphadenopathy at the porta hepatis (Fig. 5.15A,B).

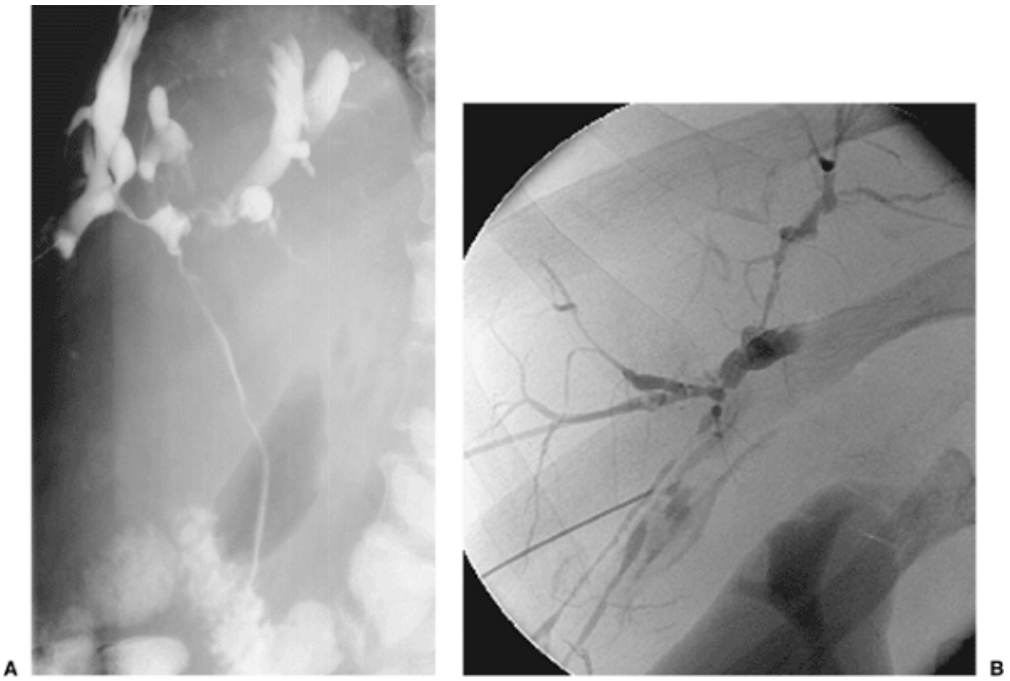


▪ **Figure 5.13** Percutaneous transhepatic cholangiogram in a patient with pancreatic carcinoma and jaundice. There is dilatation of the extrahepatic biliary system with abrupt tapering at the head of the pancreas (*arrow*) and mild intrahepatic ductal dilatation (left greater than right).



▪ **Figure 5.14** Percutaneous transhepatic cholangiogram and biliary drainage from both the right and left lobes of the liver. There is an obstructing mass (metastatic gastric carcinoma) to the porta hepatis, with obstruction of all bile ducts at this level.

When an obstruction is seen involving the extrahepatic bile ducts or the lobar bile ducts at the porta hepatis, the puncture needle is exchanged over a guide wire and an external drainage catheter is placed. An 8- or 10-Fr drainage catheter works adequately for external biliary drainage in most cases unless the bile is purulent or excessively thick, in which case a 12-Fr drainage catheter may be used. External drainage typically concludes the procedure for the day. Additional procedures that may require successful passage across the biliary obstruction, such as external–internal catheter drainage (Fig. 5.16), or internal stent placement, are best done after bile has been drained for 1 or 2 days and the risk of biliary sepsis has passed. Furthermore, external drainage for this period allows the biliary tree to decompress and return to a more normal caliber. This also reduces the time the interventionalist must spend attempting to pass the obstruction and helps limit catheter manipulations and both the patient's and the physician's radiation exposure.



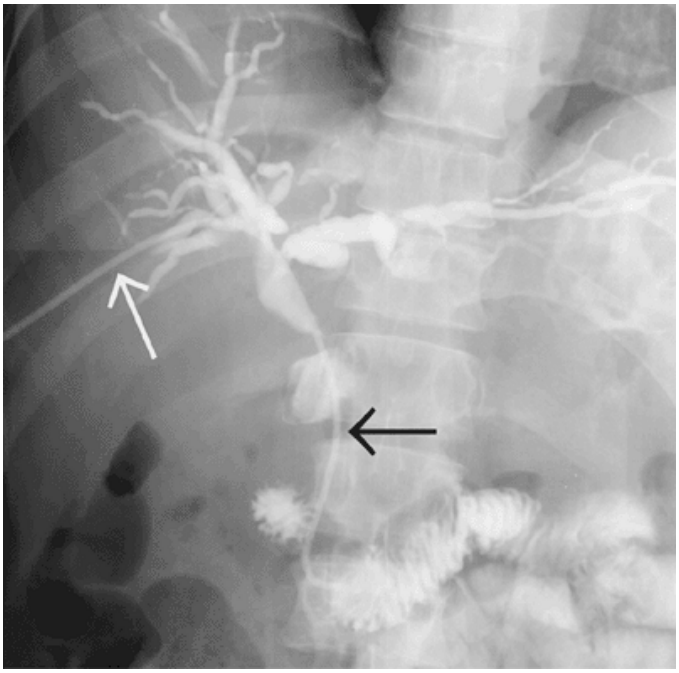
▪ **Figure 5.15** Examples of intrahepatic biliary strictures seen on percutaneous transhepatic cholangiogram. **A:** Cholangiocarcinoma involving the right and left intrahepatic bile ducts just above their confluence, with dilatation of the biliary tree in the periphery. **B:** Sclerosing cholangitis with strictured and irregular intrahepatic biliary radicals seen in many areas.

Liver Biopsy

Two methods are used to biopsy the liver—transabdominal biopsy and transvenous biopsy. The transabdominal approach is preferred in most situations because it can be

used to sample diffuse and focal liver disease and is generally quite safe and effective.

Transvenous liver biopsy is usually reserved for patients who have diffuse liver disease and comorbid conditions that make transabdominal biopsy unsafe.



▪ **Figure 5.16** Percutaneous transhepatic biliary drainage in a patient with an obstruction at the middle of the common bile duct. Both the *arrows* point to the 8-Fr drainage catheter that passes into the liver, through the right biliary system, down the common bile duct, and terminates in the duodenum. This type of catheter can be used for external drainage or capped for internal drainage.

Transabdominal Liver Biopsy

Transabdominal liver biopsy requires a needle to be passed through the skin, across the peritoneum, through the liver capsule, and into the substance of the liver. It can be performed "blind" without any imaging when there is diffuse liver disease, but the addition of ultrasound guidance ensures the safety and effectiveness of the procedure with minimal additional cost. When focal lesions of the liver are biopsied, imaging is essential. If the lesion cannot be visualized with ultrasound, the procedure may need to be performed with CT guidance. Lesions as small as 0.5 to 1.0 cm can be biopsied with this guidance (14,15,16).

The biopsy may be performed using small gauge needles (21 or 22 ga) to aspirate a sample for cytology. The presence of a qualified cytopathologist at the time of biopsy will help ascertain that adequate diagnostic material has been collected. Alternatively, a variety of cutting needles with spring-loaded handles may be used to obtain a core of the liver for histology, which can be transferred to fixative (such as 10% buffered formalin) and analyzed with various staining techniques. This is particularly important for the biopsy of certain lesions, such as lymphoma and cholangiocarcinoma, in which adequate sampling and sample preparation are crucial in arriving at a diagnosis. When these types of malignancies are suspected, special staining techniques can be used to improve the diagnostic accuracy. Overall, the accuracy of achieving the final diagnosis for image-guided biopsy ranges from 90% to 100% for core biopsies and 60% to 84%

for fine-needle aspirates (17,18).

The risks of transabdominal liver biopsy include intraperitoneal bleeding, hematobilia, pneumothorax (for right-lobe biopsies when the needle is passed through the base of the lung), and arteriovenous (AV) fistula formation. Bleeding can be avoided by taking a number of steps before and during the biopsy procedure. Correction of any coagulation abnormalities is essential. When ascites is present there is an increased likelihood of intraperitoneal hemorrhage, so postbiopsy embolization of the transhepatic tract and/or preprocedure drainage of ascites should be considered. All efforts should be made to ensure that the patient avoids deep respirations during the passage of the biopsy needle because this will help avoid liver laceration. If possible, the patient should be counseled to breathe shallowly if the biopsy requires more than 20 seconds or breath holding if the biopsy can be performed in less than 20 seconds. Postprocedure monitoring will reveal signs of bleeding, such as tachycardia, hypotension, and prolonged pain. We typically check the hematocrit 4 to 6 hours after liver biopsy and compare the results to the prebiopsy hematocrit. A drop of more than 2% to 3% should prompt further evaluation.

In a series of 853 ultrasound- and CT-guided liver biopsies, the rate of significant complications was 0.3% (19). The overall mortality rate for transabdominal biopsy is in the range 0.004% to 0.031% (20,21).

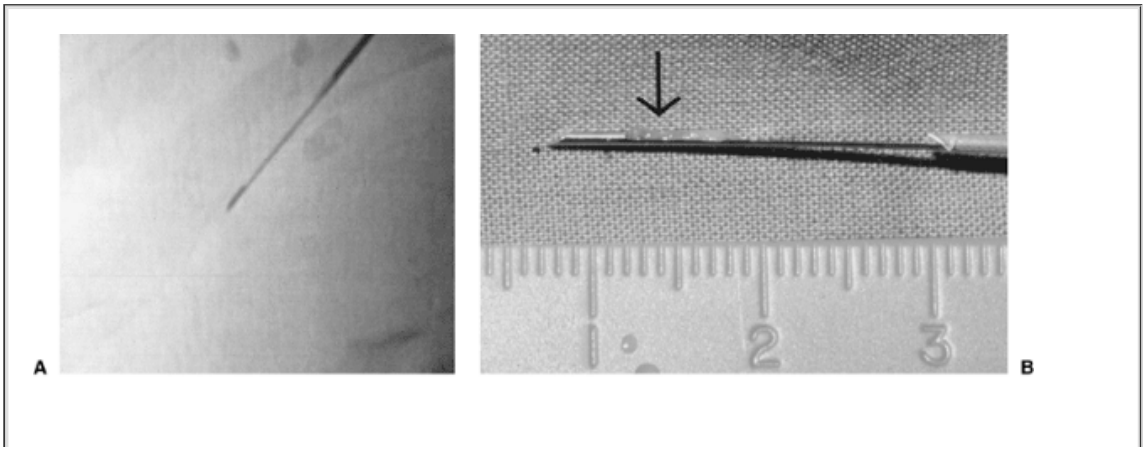
Transjugular Liver Biopsy

For patients who need liver biopsy and have comorbid conditions, such as coagulopathy (thrombocytopenia or elevated prothrombin time), ascites, or morbid obesity, or who are undergoing hepatic venography, transvenous liver biopsy is a reasonable alternative to the transabdominal approach. Over the last few years a variety of new biopsy needles have been developed that offer advantages over the original beveled 15-ga Colapinto needle.

Transjugular liver biopsy (TJLBx) was originally described in 1973 by Joseph Rosch (22), however, it was not until the 1980s that this technique was used commonly. This technique assumes that passage of the biopsy needle may induce bleeding, but because the bleeding will occur into the hepatic vein (the site where the biopsy needle enters the liver), blood will enter the vascular system without clinical consequence. The theoretic advantage of this approach has been confirmed in thousands of TJLBxs.

TJLBx needles can rarely be directed with accuracy into focal hepatic lesions because the needles are

relatively cumbersome and few hepatic lesions can be seen under fluoroscopy. Therefore, TJLBx is reserved for patients with diffuse liver disease, including cirrhosis, BCS, veno-occlusive disease, and infiltrative and metabolic processes of the liver such as storage diseases, hemochromatosis, and Wilson disease. On occasion, TJLBx can be used to perform biopsy on a large centrally located tumor within the liver.



▪ **Figure 5.17** Transjugular liver biopsy (TJLBx). **A:** TJLBx needle placement immediately before obtaining a core biopsy. **B:** Partial core within the cutting chamber of the needle (*arrow*).

TJLBx is typically started with an internal jugular vein puncture, either right sided or left sided. If necessary, the external jugular vein can be accessed. A catheter is negotiated through the superior vena cava, right atrium, and IVC, under fluoroscopic guidance. Then, one of the hepatic veins is entered, confirming the location of the catheter by hepatic venography. The catheter is exchanged over a guide wire for the biopsy system, and a puncture is made from the hepatic vein into the hepatic parenchyma (Fig. 5.17A). The needle is withdrawn and the specimen is inspected (Fig. 5.17B) and then transferred from the biopsy needle to the fixative for ultimately delivering to the laboratory.

A recent advance, the Quick-Core biopsy needle (Cook Inc., Bloomington, IN), has improved the yield from TJLBx. This device is a spring-loaded cutting needle that gives reliable core biopsy samples for histology while maintaining the safety of the biopsy procedure (23,24).

Although the incidence of TJLBx-related complications in this high-risk group is low, there are nevertheless some procedures in which the biopsy needle inadvertently traverses the liver capsule. The rate of intraperitoneal hemorrhage has been reported as 0.35% in TJLBxs from Colapinto's series of 2,271 cases (25). Yet, with careful patient selection, preprocedure review of imaging studies, attention to procedural detail, and postprocedure patient monitoring, TJLBx has become a safe method for obtaining liver tissue from patients who are considered to be at risk for transabdominal liver biopsy.

Angioplasty and Stenting

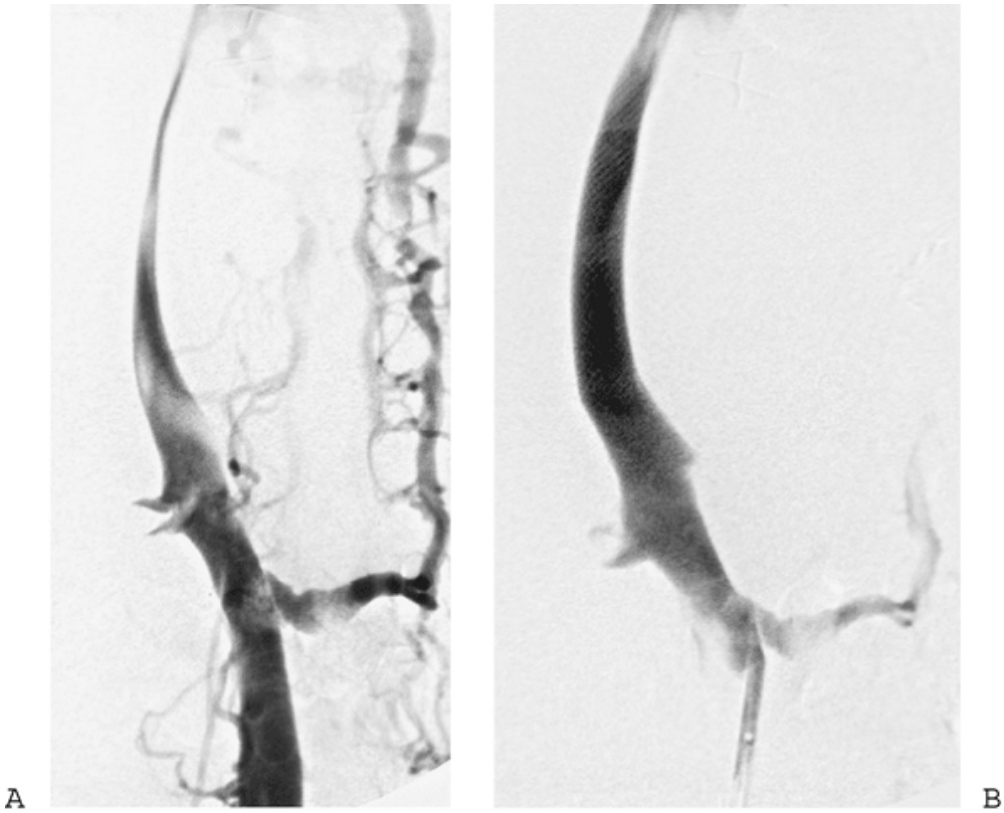
Angioplasty was developed in the 1960s as a minimally invasive approach for treating peripheral atherosclerotic disease. By the mid-1970s, the angioplasty balloon that we use today was developed, permitting vascular dilatation to a much greater diameter than was possible with earlier systems. Further refinements of angioplasty balloons and techniques have led to contemporary balloons that can withstand inflations to a pressure of 20 atm or greater and resist puncture during stent deployment.

Stents were conceived in the 1960s but not introduced for human use until the late 1980s. The original two stents were the Palmaz balloon-expandable stent and the self-expanding Gianturco Z-stent. Both types were used for creation of early TIPS shunts, and both types of stents continue to be used for hepatobiliary indications. The last decade has seen the introduction of a number of new stents, including the Wallstent, the Smart stent, the Zilver stent, and the Luminexx stent to name but a few devices. There are many hepatobiliary uses of angioplasty balloons and metal stents today. These include creation and revision of TIPS shunts, stenting of bile duct occlusions, angioplasty and stenting of hepatic vein and IVC occlusions related to BCS and tumors, and angioplasty and stenting of vascular anastomotic stenoses in the transplanted liver.

Arterial Angioplasty in Hepatobiliary Disease

Hepatic arterial stenoses after liver transplantation occur in up to 11% of cases and typically involve arterial anastomosis (26). Stenosis may lead to liver ischemia, cholangitis, liver necrosis, and ultimately transplant failure even in the absence of secondary arterial occlusion. If detected early, these stenoses can

be dilated with technical success, thus averting the need for surgical revision of the anastomosis (27,28). Once a transplant hepatic artery stenosis progresses to thrombosis, most patients will require retransplantation (29). There are no clear indications for stenting of transplant-related arterial stenoses; however, in the appropriate setting, conversion of a failed angioplasty to a successful stenting would seem appropriate.



▪ **Figure 5.18** Patient with inferior vena cava (IVC) obstruction due to cystic gastric sarcoma metastases. **A:** The IVC is diffusely stenosed, and there are multiple collaterals that communicate with the azygos venous system. **B:** After the IVC has been dilated and stented, there is direct blood flow into the right atrium and nearly complete cessation of flow into the azygos system.

Venous Angioplasty and Stenting in Hepatobiliary Disease

Malignant tumors of the liver can compress and/or invade the IVC, leading to massive lower extremity edema. When IVC obstruction involves the hepatic veins or a portion of the IVC at or above the hepatic veins, the patient may also develop ascites. Angioplasty alone is of little use because elastic recoil of the tumor causes recurrent IVC occlusion. Stenting of the IVC, however, can maintain the patency of the IVC and palliate the symptoms of IVC obstruction (Fig. 5.18A,B). Typically, self-expanding stents such as the Gianturco Z-stent and Wallstent have been used because they are less prone to migration and deformation compared to balloon-expandable stents. The reported series on stenting of the IVC are small, but the clinical success reported by Furui et al. in a series of 16 patients with IVC obstruction due to malignant tumors of the liver who were treated with Gianturco Z-stents was 100%, with no symptomatic recurrence at a mean follow-up of 3.2 months (30). Entwisle et al. also reported

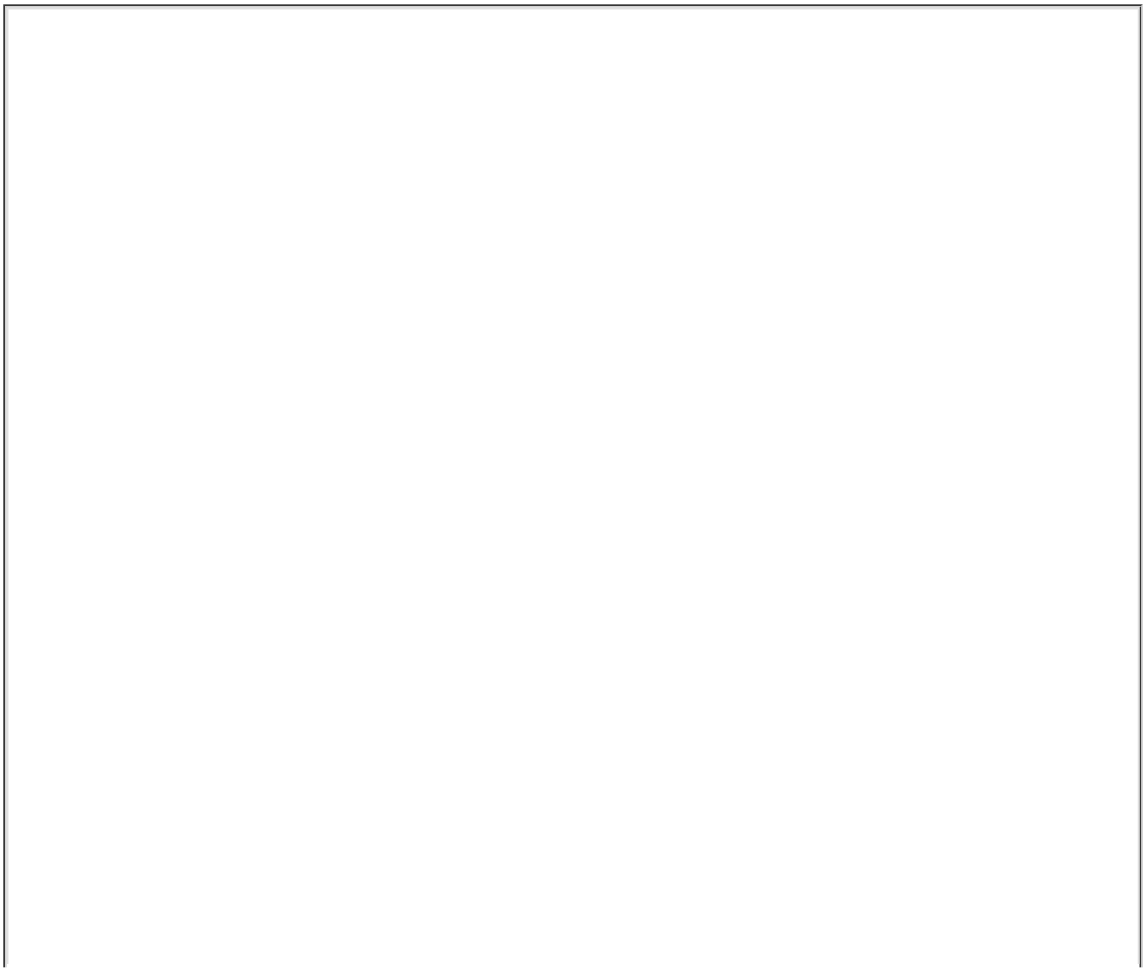
success in using the Wallstent to treat malignant IVC obstruction (31).

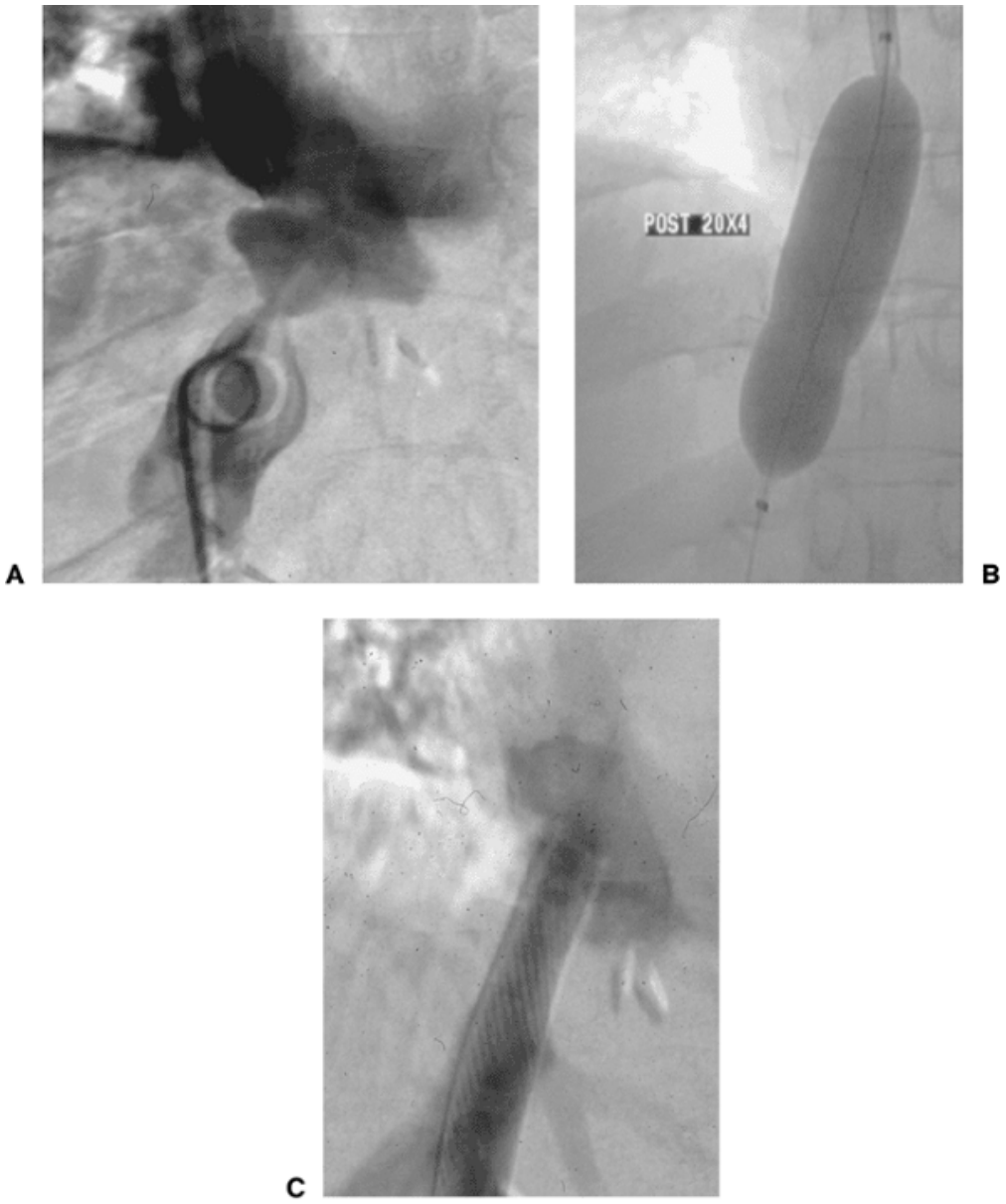
Balloon dilatation and stenting of posttransplant IVC stenosis after liver transplantation (Fig. 5.19A, B and C) has been reported in a small series (32,33,34). These techniques may help patients undergoing liver transplantation avoid surgical revision or retransplantation and should be considered as the first option when hemodynamic IVC stenosis is detected. The only caveat about stenting for this indication is that once placed in the IVC, the stent may complicate the surgeon's approach if retransplantation becomes necessary. In general, once the decision to place a stent is made in concert with the surgeon performing the transplantation, a Wallstent is preferred because it is easily cut with a scissor and, if necessary, the stent wires can removed when imbedded in tissue. The stent wires in a Gianturco Z-stent are difficult to cut and harder to extract once entrapped in tissue.

BCS represents hepatic venous occlusion that often involves the intrahepatic IVC. Venographically, there are many presentations, such as occlusive and nonocclusive thrombus in the hepatic veins, as well as webs in the hepatic veins and IVC. Many of the lesions seen in BCS can be treated using angioplasty and stenting with a high degree of success. The goal is to improve the outflow of blood from the liver and retard the process of hepatic necrosis and fibrosis.

A number of small series have described angioplasty and stenting for IVC occlusions due to BCS. In one report, 32 patients were treated using angioplasty with or without stenting. Technical success for angioplasty

and for stenting was 100%. The primary patency rate for the target stenosis or occlusion for angioplasty was 50%, and for stenting the primary patency rate was 88% (35). Another report described the use of the Wallstent to treat obstructions of the hepatic and suprahepatic IVC, with no restenoses reported in all ten patients (36).





▪ **Figure 5.19** Inferior vena cava (IVC) stricture after transplantation, with angioplasty and stenting. **A:** There is a focal IVC stricture just above the level where the hepatic veins enter the IVC. **B:** A 20-mm angioplasty balloon is used to predilate the stricture. **C:** A Wallstent is placed in the IVC and supports the angioplasty so that blood flow into the right atrium is no longer impeded.

The hepatic vein strictures and occlusions in BCS have also been approached with angioplasty and stenting techniques on a case-by-case basis. It is possible to stent stenoses of the hepatic veins (37) or to recanalize hepatic vein occlusions to "reconnect" the hepatic veins using a stent to support the postangioplasty channel that is created (38) (Fig. 5.20A,B).

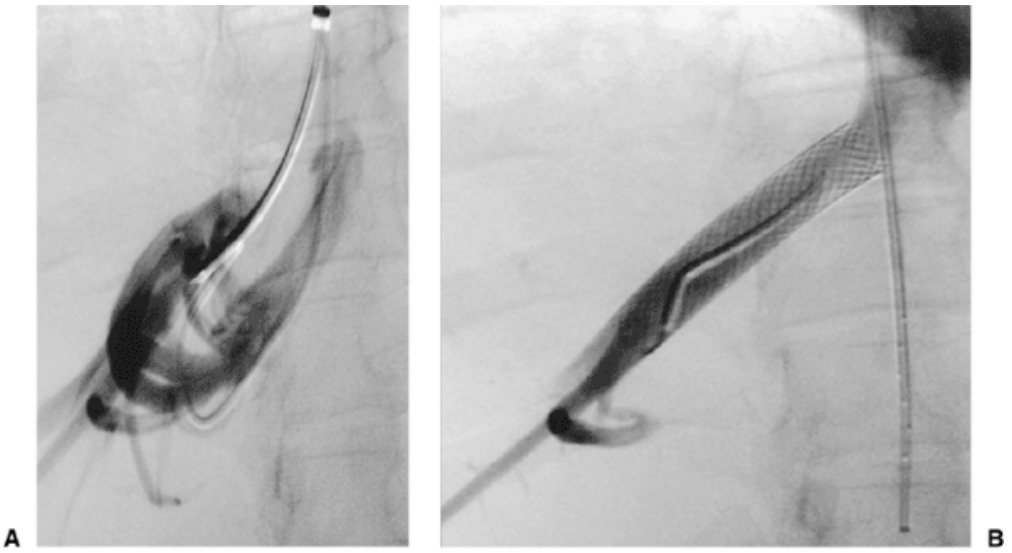
Portal vein strictures can be due to surgery (including transplantation), pancreatitis, or incomplete portal venous thrombosis. Angioplasty (39) and stents (40) have been used successfully to treat these lesions.

Transjugular Intrahepatic Portosystemic Shunt

The TIPS procedure represents the culmination of many percutaneous catheter-based techniques, including CO_2 venography, transvenous pressure measurements, angioplasty techniques, and stenting.

Biliary Balloon Dilatation (Cholangioplasty) and Stenting

Biliary strictures can be divided into benign and malignant types. The percutaneous management of these two types of lesions is distinctly different.



▪ **Figure 5.20** Reanastomosis of the right hepatic vein in a patient with Budd-Chiari syndrome. (Same patient as in Fig. 5.11.) **A:** A puncture is made from the stump of the right hepatic vein into the remaining intrahepatic portion of the right hepatic vein. **B:** After the puncture reconnects the right hepatic vein, the obstruction is balloon dilated and stented, restoring flow from the right hepatic vein into the inferior vena cava.

Benign biliary stricture dilatation

Benign strictures are typically iatrogenic (i.e., caused during surgical bypass, laparoscopic cholecystectomy, common bile duct exploration, or liver transplantation) or rarely due to underlying stone disease or adjacent inflammation such as pancreatitis. Although it is usually possible to dilate a benign biliary stricture, the results are variable. Citron and Martin (41) described 100% technical success rate for the dilatation of intrahepatic biliary strictures and 92% for the extrahepatic biliary tree strictures above the pancreas. The results were poor for intrapancreatic biliary strictures, with only a 33% success rate. The overall restenosis rate, excluding the intrapancreatic strictures, was 29%, which compares favorably with a restenosis rate of 20% to 22% from surgical series (42,43) and other interventional radiology series that reported 20% to 34% recurrence (44,45,46). It is currently the practice of most interventional radiologists to dilate biliary strictures to a diameter of 6 to 10 mm and then leave a biliary drainage catheter across the site for several months. This provides support to the dilated stricture during healing and maintains access for cholangiography before catheter removal to confirm an acceptable result at the dilated

segment.

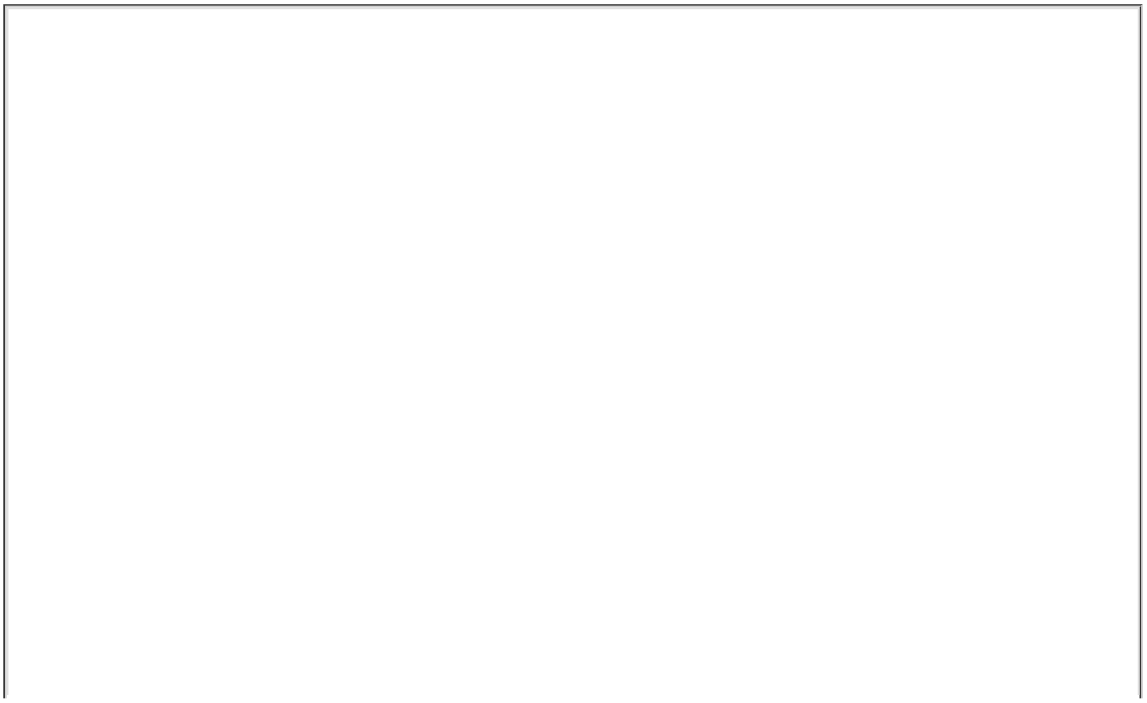
Biliary manometry can be used to assess the likelihood of successful stricture dilatation. Savader et al. (47) found that biliary manometry was a useful tool in predicting the durability of the intervention. The procedure involved a simple pressure measurement in the bile duct a mixture of contrast and saline was infused at a step-up rate every 5 minutes until a rate of 20 mL/minute was reached. Patients whose intrabiliary pressure remained less than 20 cm H_2O during all infusion rates without symptoms of pain, nausea, vomiting, or chills were considered to have "passed" the perfusion test. Patients who passed the perfusion test had a 0.9 probability of patency at 3 years, whereas patients who failed had a 0.45 probability of patency at 3 years. Alternatively, patients could be managed by "clinical trial," converting the supportive biliary catheter to one that resided above the site of dilatation and then capping the tube for several weeks so that it would serve as an access to the biliary tree if necessary. If the patient had nausea, vomiting, fever, chills, bile leakage, or discomfort flushing the catheter, they were considered to have failed the clinical trial. If the patient remained asymptomatic, the tube was removed. With either manometry or clinical trial, patients who were likely to have success and those who were likely to fail after biliary dilatation or surgery were identified.

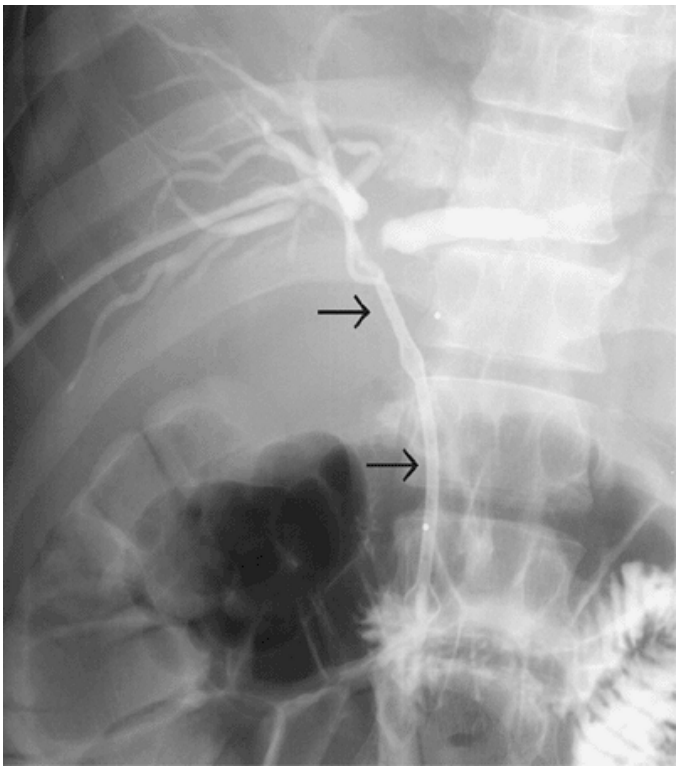
Although the placement of a percutaneous biliary catheter is an essential component of visualizing transhepatic cholangioplasty of benign strictures, there has been no proof that the placement of a permanent metallic stent offers any patency advantage despite the demonstration of a typically superb immediate technical result. In fact, the placement of a metallic biliary stent is usually avoided when treating benign strictures because the durability of treatment is measured in years, whereas metallic biliary stents often occlude within a few months because of a variety of causes, as described in the following text.

Malignant stricture dilatation

Unlike many benign biliary strictures, malignant strictures do not respond favorably to balloon dilatation

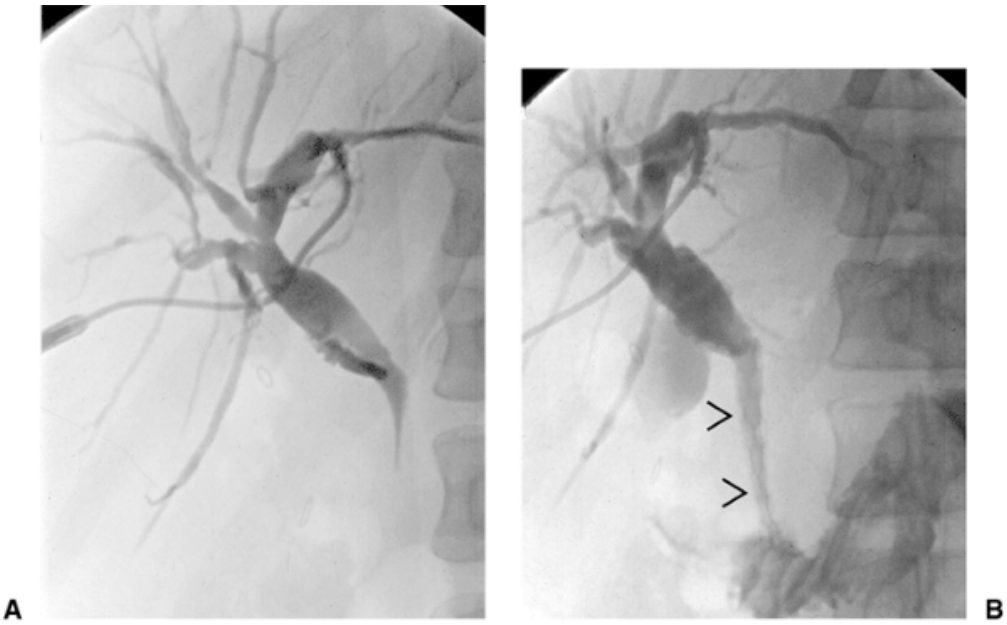
alone because of the mass effect and elasticity of the tumor and adjacent tissues such as enlarged lymph nodes. To achieve an acceptable technical result after balloon dilatation of a malignant biliary stricture, addition of a stent is necessary.





▪ **Figure 5.21** Percutaneously placed internal biliary stent. The stent (*arrows*) resides in the extrahepatic bile duct, with its distal tip entering the duodenum. These tubular plastic stents are particularly prone to occlusion. Furthermore, and as seen in this case, it may be difficult to position this stent accurately if there is a lesion in the porta hepatis or high extrahepatic biliary tree.

Many different stents, both plastic (Fig. 5.21) and metal (Figure 5.22A,B), have been developed for the biliary tree, and they all afford an excellent technical result at the end of the procedure. Unfortunately, these stents are also fraught with early (<30 days) and late (>30 days) complications. In a comparative series, metallic stents had an early occlusion rate of 18%, which was nevertheless better than plastic stents, with a 50% early occlusion rate (48,49). Therefore, endoscopically placed metal stents have a distinct advantage over plastic stents in the first month. A similar comparison was done for percutaneously placed stents, with an early obstruction rate of 19% versus 27% for metal versus plastic stents (50). The median patency of percutaneously placed metal stents was much greater than of plastic stents—272 days versus 96 days, respectively ($P < 0.01$). Nevertheless, stent occlusion remains a problem even for metal stents. Occlusion occurs because of bile salt encrustation, inspissated gastric debris, tumor ingrowth, and epithelial hyperplasia. Therefore, the use of indwelling metallic stents is typically reserved for patients with a life expectancy less than a year, such as those with unresectable pancreatic carcinoma or metastatic adenocarcinoma. For patients with slowly growing malignancy, such as many of those with cholangiocarcinoma, internal metal stents are problematic, and we usually recommend internal–external biliary catheters that can be easily changed every 3 to 6 months over a guide wire using a percutaneous transhepatic approach.



▪ **Figure 5.22** Pre- and post–stent images in a patient with pancreatic carcinoma. **A:** There is abrupt tapering of the distal common bile duct because of obstruction from the pancreatic head mass. **B:** A self-expanding metal biliary stent has been placed, restoring continuity between the biliary system and the duodenum (*arrowheads*).

In summary, benign strictures above the level of the pancreas typically respond to balloon dilatation, and placement of a metallic indwelling stent should be avoided. The cholangioplasty site, however, should be internally supported during the healing process by placing a temporary plastic drainage catheter or

an endoscopically removable plastic stent. Malignant biliary strictures almost always require internal stent placement, and metal stents are preferred over plastic stents because of a relative patency advantage. Nevertheless, all internal stents are prone to occlusion, and internalized biliary stents are recommended for patients with limited life expectancy or those who are willing to accept the likelihood that additional procedures will be needed to treat an obstructed internalized stent.

Thrombolytic Therapy

The use of thrombolytic agents in hepatobiliary disease is not common but can offer dramatic nonsurgical cure for acute portal vein thrombosis (50,51). Thrombolytic agents such as tissue plasminogen activator (tPa) can be infused into the mesenteric venous clot by direct portal vein access, as described previously for transhepatic portography. Alternatively, thrombolytic agents have been delivered into the superior mesenteric artery with transcapillary passage into the venous circulation (52).

Eagerness to treat mesenteric vein thrombosis with thrombolytic agents, however, should be tempered until the cause for thrombosis has been determined and the risk for bleeding while on thrombolytic treatment assessed. It is important to recognize that a number of conditions may cause portal vein thrombosis, and accurate diagnosis and treatment may be necessary before initiating thrombolytic therapy. These conditions include primary disorders of thrombosis, myeloproliferative disorders, and dehydration. Multiple concurrent factors may lead to portal vein thrombosis (53).

Embolotherapy

With the ability to catheterize fourth- and fifth-order hepatic arterial branches, embolotherapy techniques are used frequently to treat hepatic artery injury and AV lesions of the liver. When embolotherapy is coupled with transcatheter delivery of chemotherapeutic agents or radioactive particles, the combined effect offers aggressive ablation of liver tumors. Transhepatic portal vein embolization (PVE) is evolving as a method to hypertrophy a portion of the liver that will remain in the patient after partial hepatic resection.

Embolotherapy of Nonmalignant Lesions of the Liver from the Hepatic Artery

Embolic agents used in the hepatic artery include particulate materials (polyvinyl alcohol [PVA], polyacrylamide microspheres, microfibrillated collagen, and gelfoam particles and pledgets), embolic metal coils, oily agents (Ethiodol and Lipiodol), adhesives, autologous clot, and detachable balloons. Each type of agent is selected for a particular property such as the size of vessel it will occlude, flow rate across the lesion (especially important when treating high-flow AV shunts), and the need for short-term versus permanent occlusion.

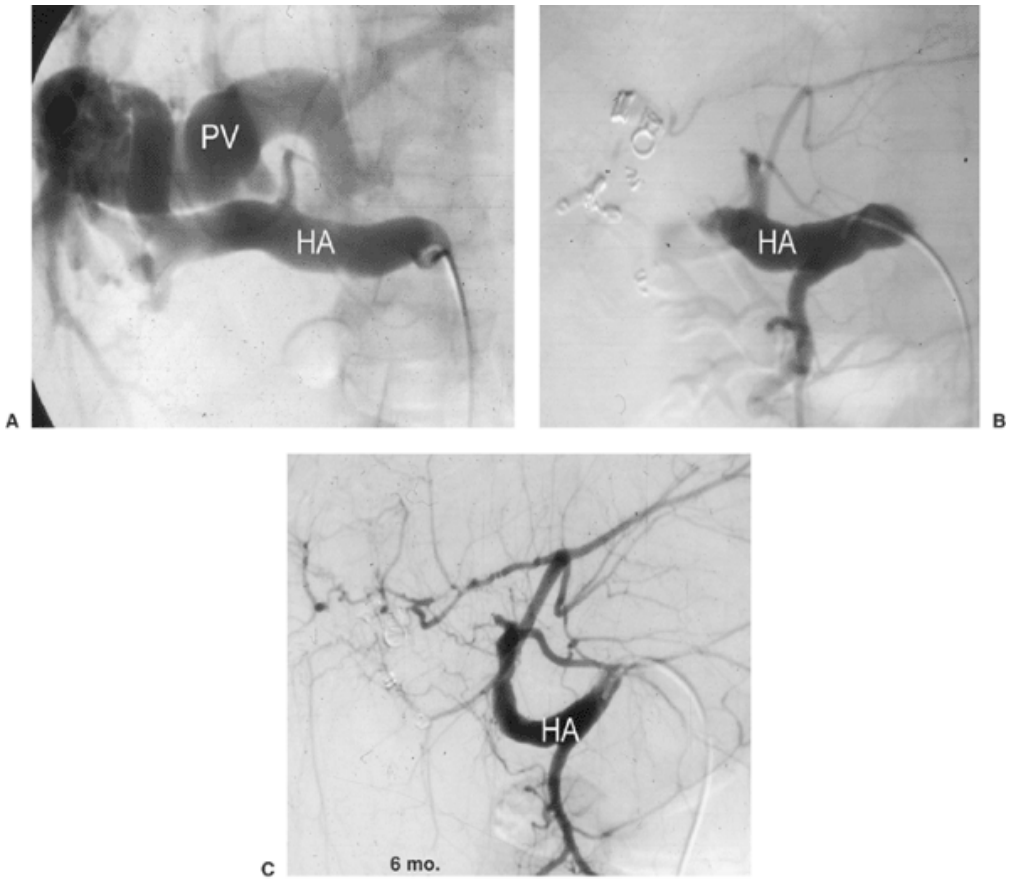
AV lesions of the liver are rare. When an AV shunt is guided from the hepatic artery into the portal venous system there may be signs of portal hypertension such as ascites, formation of varices, variceal hemorrhage, or encephalopathy. Venovenous shunts can develop, connecting the portal venous system to the systemic system, with clinical manifestations of encephalopathy, seizures, and heart failure.

Trauma is a well-recognized cause of hepatic AV fistula formation. Using particulate emboli and coils, it is possible to occlude all branches, leading to a complex AV fistula or AV malformation, and provide a durable cure (Fig. 5.23A–C). An example of a venovenous shunt is the persistent ductus venosus, a rare condition in which a persistently patent ductus venosus shunts blood from the left portal vein to the LHV. Careful delivery of embolic coils can permanently occlude this shunt and restore portal flow to the liver (Fig. 5.24A,B).

Aneurysms and pseudoaneurysms of the hepatic artery are uncommon. They may be due to hepatitis, trauma, pancreatitis, tumor, or iatrogenic causes (Fig. 5.25A,B) such as biopsy or biliary drainage. Pseudoaneurysms can also be related to the disruption of the anastomosis of the hepatic artery during liver transplantation. Many of these lesions can be managed with therapeutic transcatheter embolization.

Transarterial Chemoembolization of Malignant Tumors in the Liver

Delivery of embolic agents along with chemotherapy, termed *TACE*, has become one of the methods for palliation of hepatocellular carcinoma, cholangiocarcinoma, hepatic metastases from carcinoid tumors, islet cell tumors, and ocular melanoma. Many chemoembolic concoctions have been promoted over the years. Perhaps the most widely employed techniques call for embolic PVA particles mixed with a three-drug regimen that includes doxorubicin, cis-platinum, and mitomycin-C, although there are also many reports on the use of only one chemotherapeutic agent such as doxorubicin or cis-platinum. The addition of an oily contrast agent (Ethiodol or Lipiodol) is believed to enhance the embolic effect of particles by occluding flow at the sinusoidal level (54,55). The use of oily contrast also helps the interventional radiologist during the administration of the chemoembolic mixture because these oily agents are densely radioopaque and, therefore, easily seen as they enter the hepatic arterial system and tumor (Fig. 5.26A,B).



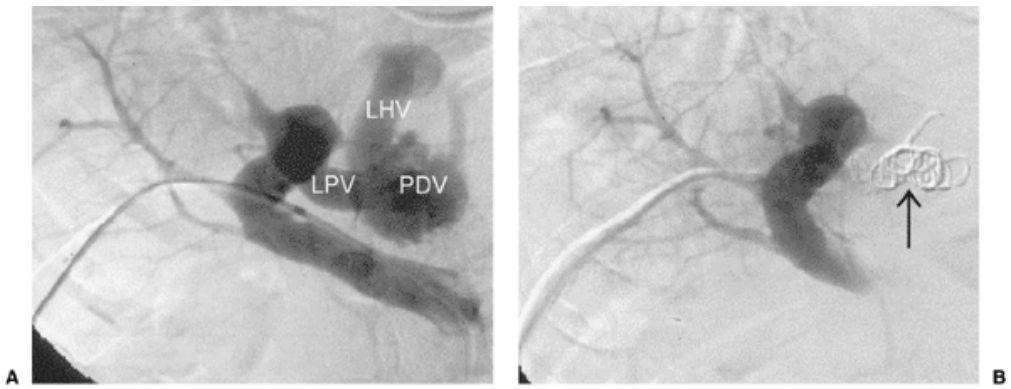
▪ **Figure 5.23** Arteriovenous fistula (AVF) and embolization. This young man sustained a liver laceration 10 years earlier and presented with signs of portal hypertension including ascites and varices. **A:** There is a complex AVF connecting the right hepatic artery (HA) to the right portal vein (PV), with contrast entering the main PV. **B:** Occlusive metal coils and small gelfoam pledgets have been used to perform therapeutic embolization of the AVF. There is no residual PV filling. **C:** Six-month follow-up angiogram. The AVF remains thrombosed. The main HA has returned to near-normal caliber. Intrahepatic collaterals have developed from the left hepatic arterial system to the right during this interval. The patient no longer evidenced signs or symptoms of portal hypertension.

A number of investigations have been performed demonstrating the effectiveness of this multitherapy embolic regimen in the treatment of hepatocellular carcinoma (56,57,58,59). Most reports of TACE for the treatment of unresectable hepatocellular carcinoma note tumor regression in many patients and prolonged periods of tumor stability with minimal treatment-related toxicity. Survival of these patients after TACE is prolonged compared with historical survival data for patients not treated in this way (56,58).

Randomized data has recently become available. Three prospective randomized trials and one meta-analysis confirm the survival advantage of TACE compared with supportive care or bland particle embolization (no chemotherapy) for unresectable hepatocellular carcinoma. Llovet et al. (60) randomized 112 patients, with most of them being infected with hepatitis C virus and classified as Child-Pugh A (70%), into one of three groups—TACE, bland embolization (no chemotherapy), and supportive

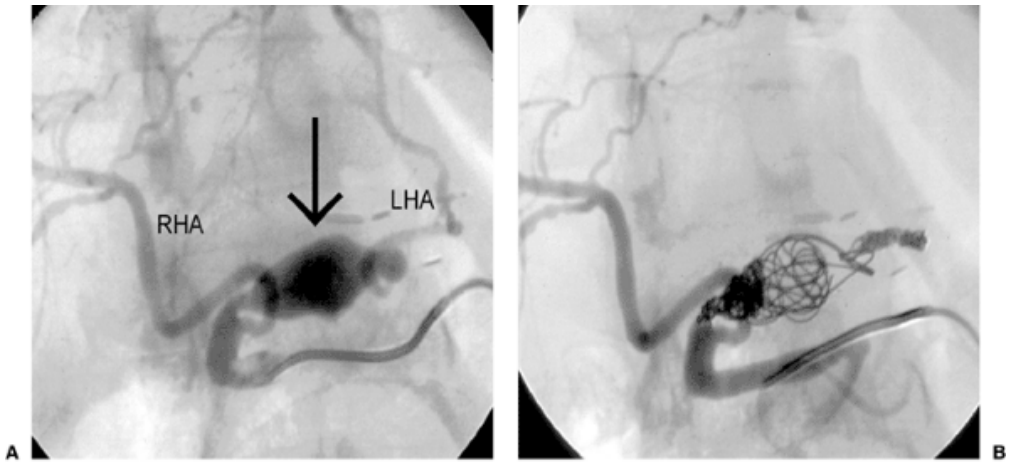
medical therapy. The median tumor size was 5 cm (range 3.9 to 6 cm) and patients with portal vein invasion were excluded. This trial was halted early when a clear survival advantage was seen with TACE compared to supportive therapy. The 1-, 2-, and 3-year survival rates for TACE were 82%, 63%, and 29%, respectively, while the survival rates for supportive therapy were 63%, 27%, and 17% at 1, 2, and 3 years, respectively (*P* = 0.009). Patients receiving TACE also had better survival than those who underwent treatment of their tumor using bland

particle embolization (no chemotherapy). In the study by Llovet et al. the TACE regimen included doxorubicin and Lipiodol while bland particle embolization was performed with gelfoam particles.



▪ **Figure 5.24** Patent ductus venosus (PDV). This patient presented with right-sided heart failure and had several recent seizures. Ammonia levels were elevated. **A:** Transabdominal-transhepatic portal vein catheterization into the right portal vein. Portal venography demonstrates dilatation of the left portal vein (LPV), the presence of a PDV, and flow into the left hepatic vein (LHV). **B:** Transcatheter embolization of the communication branch of the LPV and the PDV has been successful. This patient's cardiac dynamics improved and his ammonia levels normalized.

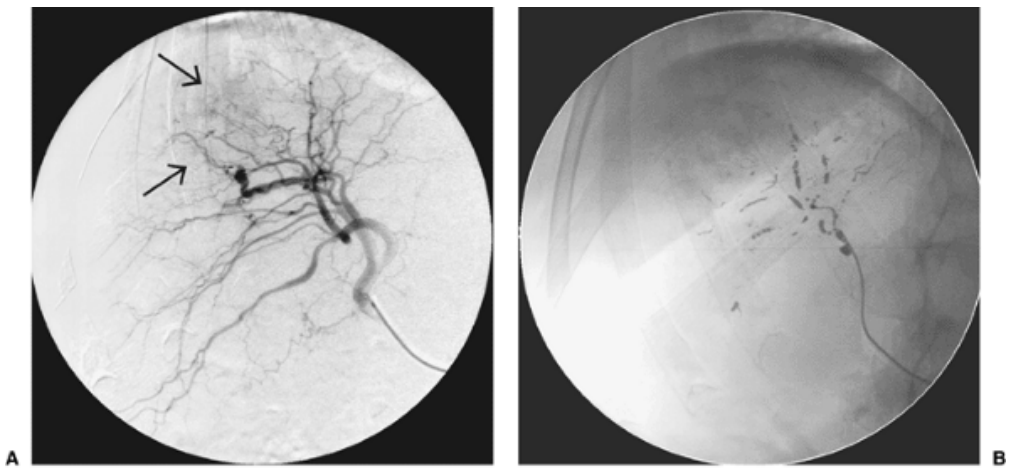
Lo et al. (61) reported a series of patients with unresectable hepatocellular carcinoma who were treated with a cis-platinum and Lipiodol TACE regimen. Most patients were positive for hepatitis B virus (HBV), mean tumor size was 7 cm (range 4 to 14 cm), and patients with portal vein invasion were allowed into the study. Patients were randomized between TACE and supportive therapy. The 1-, 2-, and 3-year survival in the TACE group was 57%, 31%, and 26%, respectively, while survival in the supportive therapy group was 32%, 11%, and 3%, respectively.



▪ **Figure 5.25** Catheterization of the proper hepatic artery followed by therapeutic transcatheter embolization of a left hepatic artery pseudoaneurysm. **A:** This patient had liver transplantation several years earlier, and a hepatic artery pseudoaneurysm was incidentally noted on a computed tomography (CT) scan obtained for other reasons. Hepatic arteriogram showing that the pseudoaneurysm (*arrow*) arises from the left hepatic artery (LHA). The right hepatic artery (RHA) arises proximal to the pseudoaneurysm. **B:** Therapeutic transcatheter coil embolization of the pseudoaneurysm and the short segment of the LHA leading into and away from the pseudoaneurysm. Intrahepatic arterial collaterals from right to left were seen immediately after the occlusion of the proximal LHA.

Yuen et al. (62) reported a comparative trial of 96 patients with hepatocellular carcinoma, with a preponderance of patients who were HBV positive,

randomized between TACE (cis-platinum and Lipiodol and supportive therapy. Although the median tumor size was 3 to 4 cm, the range of tumor size was quite wide and the trial included tumors as large as 20 cm diameter. Patients were mostly classified as Child-Pugh A. The median survival time of the patients receiving TACE was 31.2 months while the control group had a median survival of 14.1 months (P = 0.0126). The 1- and 2-year survival for the TACE group was 86.3% and 78.8%, respectively, while the patients in the supportive therapy group had 1- and 2-year survival of 62.5% and 50%, respectively.



▪ **Figure 5.26** Transarterial chemoembolization of a hepatocellular carcinomas. **A:** There is neovascularity in this region of the right lobe hepatocellular carcinomas (*arrows*). **B:** The injection of the chemotherapeutic agent mixed with embolic particles and Ethiodol (oily contrast material) appears as droplets on the fluoroscopic image. These droplets migrate into the tumor with blood flow until enough occlusion has reduced the flow to the point of sluggishness or stagnation.

A meta-analysis of seven randomized studies of TACE for treating unresectable hepatocellular carcinoma was recently reported by Llovet and Bruix (63). This analysis also demonstrated that TACE offers a survival benefit, with a reduced risk of death at 2 years for TACE compared with supportive therapies (odds ratio 0.42 to 0.53 depending on which trials were included in the analysis).

Although randomized studies now validate the advantages of TACE over supportive therapy, a critical review of this literature shows that there is still much work to do. There is no agreement regarding the optimal chemotherapeutic agent(s), the optimal embolic agent(s), or even whether TACE should be performed as a scheduled series of treatment or only when there is evidence of disease progression. Many of these unresolved issues are highlighted in the recent review by Reidy and Schwartz (64).

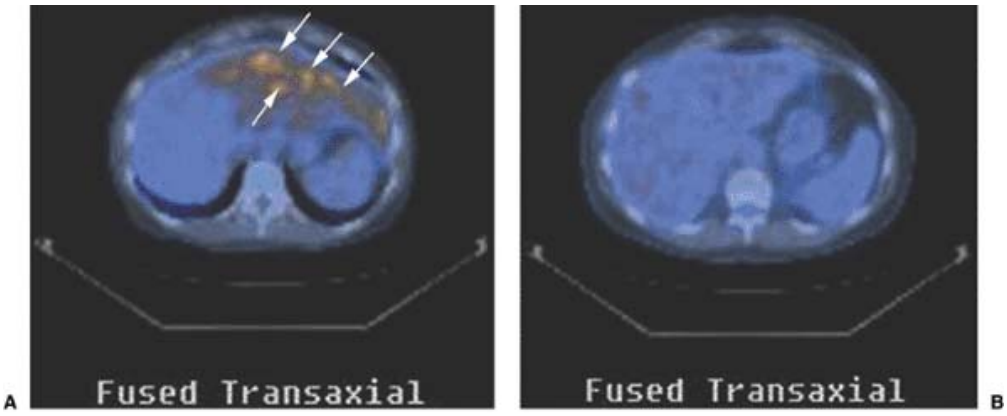
There is no randomized data on TACE for the treatment of unresectable cholangiocarcinoma; however, a recent report by Burger et al. (65) describes encouraging results in a group of 17 patients treated over a 5-year period. Nearly all patients were Child-Pugh A with a TNM staging of II or III. TACE consisted of embolic particles, Ethiodol, and a triple-drug mixture of doxorubicin, cis-platinum, and mitomycin-C. Eleven patients underwent only TACE, four had concurrent chemotherapy, and two had concurrent chemotherapy and radiation therapy. The median survival was 23 months, markedly better than historical reports of survival for patients with cholangiocarcinoma, in which the median survival was approximately 6 months and effective therapy very limited (66,67).

Hepatic metastases from carcinoid tumor respond to TACE, with many reports of tumor shrinkage and control of symptoms related to pharmacologically active metastases. When hepatic metastases from the carcinoid tumor are treated with TACE, the 5-hydroxyindoleacetic acid (5-HIAA) levels associated with the carcinoid syndrome normalize and symptoms resolve or diminish in most patients. Drougas et al. (68) reported an approximate 75% clinical response rate. Symptoms associated with carcinoid syndrome due to hepatic metastases such as diarrhea, flushing, abdominal pain, and malaise were successfully treated in most patients in this study. Additionally, biochemical markers of carcinoid metastases decreased by 60% ± 6% for 5-HIAA, 75% ± 10% for chromogranin A, and 50% ± 7% for neuron-specific enolase at 3 months (mean% ± standard error).

Symptomatic relief and tumor shrinkage have also been reported for a number of other types of pharmacologically active metastases including islet cell malignancies such as gastrinomas, glucagonomas, and

insulinomas. The reported success rate for symptom control after TACE treatment of these biochemically active metastases is reported to range from 90% to 100% (69,70,71,72,73).

Ocular melanoma metastases to the liver have been treated successfully using TACE. In a series of 30 patients, Mavligit et al. (74) reported an actuarial survival of 33% at 1 year, whereas the median survival for patients who treated by other methods is 2 to 6 months (75).



▪ **Figure 5.27** Selective internal radiation therapy (SIRT) of metastatic lung carcinoma to the liver. **A:** Fusion computed tomography/positron emission tomography (CT/PET) of a 52-year-old woman with metastatic lung cancer to the liver. The primary tumor has been removed and metastases to the liver (*arrows*) are the only identified sites of disease. **B:** Fusion CT/PET obtained 5 months after two treatments with SIRT. Whole liver treatment was performed twice, initially with 1.3 GBq of Yttrium 90 resin particles followed 8 weeks later with a second treatment using the same type of particles delivering 1.0 GBq. There was no demonstrable metastatic disease in the liver.

Finally, there has been increasing interest in use of TACE for metastatic colorectal carcinoma to the liver, which, in the United States, affects more people than the combined number of people with hepatocellular carcinoma, and hepatic metastases from carcinoid tumor, ocular melanoma, and islet cell tumors. To date, TACE appears to shrink the size of colorectal metastases, but there is still no clear survival advantage for these patients (76,77,78,79). Fortunately, other systemic and local–regional therapies have evolved for colorectal metastases to the liver. Local–regional therapies including transarterial radioembolization and radiofrequency ablation (RFA) are presented in the following two sections.

Transarterial Radioembolization of Malignant Tumors in the Liver

Intra-arterial delivery of radioactive Yttrium 90 microspheres, referred to as *SIRT*, is one of the more recent additions to the array of percutaneous ablative therapies for hepatic malignancies. Yttrium 90 is a pure β-particle emitter produced by neutron bombardment of Yttrium 89. The half-life of Yttrium 90 is 64.2 hours, and on radioactive decay it releases a β particle with the energy of 0.94 MeV and transforms to stable Zirconium 90. β-Particles released from decay of Yttrium 90 have a mean penetration of 2.5 mm into the tissue and a maximum range of approximately 11 mm. Yttrium 90 can be coupled to a carrier bead made of either resin (SIR-Spheres) or glass/ceramic (TheraSpheres) that are approximately 30 μm in diameter. When delivered through an angiographic microcatheter into the hepatic arteries that feed the liver tumor, these beads lodge in the tumor capillary bed and irradiate the surrounding tissue. Using this technique, between 5 and 50 million spheres are delivered to a tumor in the liver, depending on the type of particle, size of tumor, and dose calculations (80). Radioactive Yttrium 99 particles deliver an extremely high local radiation dose. One GBq (27 mCi) is within the range of a single therapeutic dose delivered during SIRT. Radioactive particles will deposit up to 3,000 Gy in a tumor (81).

The results of Yttrium 90 particle SIRT are impressive. In a small randomized series, the lifespan of those patients with colorectal carcinoma who had one dose of SIRT coupled with systemic chemotherapy was twice as good as that of the chemotherapy arm (82). These patients were treatment naïve and had normal liver function test results, and therefore, were not the usual patients seen in US clinical trials who had already failed prior systemic chemotherapy and had often presented with liver function abnormalities. Yet recent experience shows that even in studies of SIRT in patients who have failed systemic chemotherapy, mean survival is 10.5 months while nonresponders survive only 4 months (83,84,85). Similar results have been seen with cholangiocarcinoma, hepatocellular carcinoma, and metastatic breast, neuroendocrine, lung, and other malignant tumors in the liver (86,87,88,89). It is important to note that response may be characterized in different ways, and follow-up imaging studies may show persistent masses that have little or no residual malignant tissue. Because of this, serum tumor markers and/or imaging studies that also show tumor activity, such as CT fused with positron emission tomography (PET), may give a more reliable assessment of the success of SIRT treatment (Fig. 5.27A,B).

One major and avoidable complication of SIRT is unanticipated shunting of radioactive particles to the lungs. This may happen when there are abundant tumoral artery-to-systemic venous circuits that allow shunting of the SIRT particles around the tumor's capillary bed. The lifetime dose of radiation to the lungs is 30 Gy, and it does not take a significant number of radioactive particles in the pulmonary parenchyma to cause radiation pneumonitis, a possibly lethal complication of SIRT. Shunting of 10% to 20% of the anticipated tumor dose can induce radiation pneumonitis (there are different allowable shunt percentages for SIR-Spheres and TheraSpheres for various technical reasons).

Preliminary visceral arteriogram is performed in advance of anticipated SIRT treatment to assess shunt percentage before delivery of the SIRT particles. At the completion of this arteriogram, Technetium Tc 99m—labeled macroaggregated albumin (Tc 99m MAA) particles are injected into the target hepatic artery. Distribution of the Tc 99m MAA particles in the liver, lung, and viscera simulates the expected distribution of radioactive microspheres. Calculation of scintigraphic activity from the concentration of Tc 99m MAA in the lungs and liver is used to determine the amount of pulmonary shunting.

Other complications related to SIRT include deposition of the spheres in the arteries that supply the gastrointestinal system. Inadvertent radioactive particle embolization in the gut can result in radiation gastritis and enteritis, with possible ulceration and slow mucosal healing. Meticulous pre-SIRT angiography is required, and aberrant visceral supply to the stomach, pancreas, or large or small bowel from the hepatic arterial circulation can often be managed by branch

vessel occlusion with embolic coils or gelfoam pledgets at the time of preliminary diagnostic angiography or immediately before SIRT. Successful occlusion of aberrant visceral branches is usually confirmed during the same Tc 99m MAA distribution study that is performed to assess shunting to the lungs.

Finally, it is possible for appropriately located radioactive particles to induce damage of normal liver tissue, and overdose of particles in the liver can lead to radiation-induced liver dysfunction (90). A recent report that studied the hepatotoxicity of TheraSpheres found that patients with elevated serum bilirubin levels had a higher incidence of SIRT-related liver toxicity. There was also a correlation between higher radiation dosage and liver toxicity, although none of the patients with liver toxicity had confirmation of radiation-induced liver damage, and all toxicities resolved over time (91).

Percutaneous Needle-Directed Tumor Ablation

Having just discussed both transarterial chemoembolization and transarterial

radioablation of hepatic malignancies, it is important to mention an entirely different approach for tumor ablation—percutaneous needle-directed ablative therapies. During the last decade, a variety of ablative approaches have been used, including injection of necrosing agents (e.g., acetic acid, ethanol, hot saline), freezing techniques (e.g., cryoablation), ultrasonic methods, microwave ablation, and, most recently, the widespread use of RFA. All methods aim to deliver a necrosing blow into the tumor by open, laparoscopic, or image-guided placement of a needle or probe that can deliver the therapeutic agent directly into the tumor.

There is no substantial prospective randomized data comparing any of these techniques to each other. However, it is generally recognized that all these techniques can offer successful ablation of tumors less than 5 cm, and the smaller the tumor, the more effective the treatment. Ethanol ablation calls for delivery of 95% ethanol through a needle that has been placed with its tip in the tumor by ultrasound or CT guidance. Delivery of ethanol causes coagulative necrosis, with response rates for tumors less than 3 cm in diameter approaching 100% (92). The 5-year survival rates for patients with hepatocellular carcinomas 3 cm or less in diameter treated with ethanol is 48% (93,94,95).

Fifty percent acetic acid injection is delivered into the center of the tumor through a needle placed percutaneously. Coagulative necrosis, similar in appearance to the results seen with ethanol injection, causes tumor regression. However, for patients with up to four lesions that were not greater than 3 cm in diameter there was better survival at 1 and 2 years (100% and 92%) compared with the results for ethanol (83% and 63%). Furthermore, tumor recurrence was less frequent at 2 years with acetic acid compared to ethanol (8% with acetic acid vs. 37% with ethanol), whereas the 5-year survival rate for patients with hepatocellular carcinoma measuring 3 cm or less in diameter who were treated with acetic acid was 49%, similar to the results for ethanol (96,97).

Cryoablation uses a probe that delivers freezing temperatures to the center of the tumor and causes

tumor death by direct cellular freezing and indirectly through vascular thrombosis and tissue anoxia (98). Initial experience with this technique from a truly percutaneous approach has waned until recently as other ablative techniques have been developed, in part because of the large probe size that was required. Also, unlike RFA, the percutaneous tract cannot be cauterized with the cryoablation probe, so there is the potential for higher bleeding risk with cryoablation.

Now, however, miniaturization has led to more acceptable cryotherapy probe sizes, with diameters in the range of 2.4 to 3.0 mm. There are also some theoretic advantages of cryoablation compared with other percutaneous ablation therapies. There is no limitation from "heat sink" (as encountered with RFA, see in the following text) nor are there chemical dosing or toxicity issues (associated with acetic acid and ethanol injection). Cryoablation, therefore, may return as a viable method for percutaneous tumor ablation in the coming years.

RFA is currently the most widely used percutaneous ablative treatment for liver tumors in the United States. This procedure requires the insertion of a multitined probe into a tumor under ultrasound, CT, laparoscopic, or open surgical access. Then, an electric current is applied to the probe and a sphere of heating ensues proportional to the fourth power of the distance from the electrode. Thermal injury to cells starts at about 41°C, but most tumors in humans require temperatures of 45°C to 50°C for cell death to occur. As with ethanol and acetic acid ablation, larger tumors are more difficult to ablate than smaller ones. Until recently, tumor diameter limit for RFA was approximately 5 cm (99,100,101), but advances in probe design, as well as repositioning the probe to treat a larger volume, has made RFA a reasonable therapy

for tumors that are larger than 5 cm in diameter.

Disease-free survival at 12 to 15 months after RFA of primary and metastatic tumors of the liver without other ablative therapies was approximately 70% from two large series that studied tumors ranging from 1 to 10 cm in diameter (102,103). While this data is from laparoscopic placement of the RFA probe, similar data have been reported for percutaneous hepatocellular carcinomas 3 cm or less in diameter (104). Survival data for percutaneous RFA of hepatocellular carcinomas is comparable to those achieved with ethanol and also acetic acid, with 1- and 5-year survival of 94% and 40%, respectively. Lencioni et al. (105) treated 53 metastases in 29 patients, mostly from colorectal carcinoma. They reported a complete response in 77%, with a recurrence rate of 12%. With a mean follow-up of 6.5 months, 68% of the 53 lesions had no evidence of tumor progression.

A recent report of 1,000 RFA procedures directed at treating hepatocellular carcinoma in 664 patients confirms the effectiveness and safety of this therapy (106). There were 2,082 sessions of RFA to treat 2,140 nodules, with a mean lesion size of 2.6 cm. Of the 1,000 procedures, the major complication rate was 4.0% (40 procedures) with no treatment-related deaths. Major complications included intraperitoneal hemorrhage (four procedures), hepatic infarction in two patients, hepatic abscess in seven patients, and intestinal perforation/penetration in three patients. For the one patient with bile peritonitis, surgical and endoscopic biliary drainage was required.

From this large series of RFA for hepatocellular carcinoma, the long-term outcomes were comparable to other forms of ablative therapy, with 1-, 2-, 3-, 4-, and 5-year survival rates of 94.7%, 86.1%, 77.7%, 67.4%, and 54.3%, respectively, in a patient population in which approximately 80% of the treatment population was hepatitis C–antibody positive and 10% were hepatitis B–antigen positive. Tumor recurrence was seen in just over half the patients (165 of 306 patients) with a median time to recurrence of 1.59 years. Of the 165 patients with recurrence, 137 had three or fewer recurrent lesions and were treated with repeat RFA procedures. It is clear that RFA can be used to treat hepatocellular carcinoma with a low complication rate, and although recurrence is likely in at least half the patients, most of these patients can be retreated.

RFA for hepatic metastases is in evolution, especially given the recent interest in radioablative embolization, as well as ongoing developments in systemic therapies. Nevertheless, in the largest series to date, Lencioni et al. (107) describes the multicenter data for 423 patients with four or fewer colorectal hepatic metastases treated with RFA. The average metastasis size was 2.7 cm, with a mean follow-up of 19 months. Local tumor progression was observed in 25.15% of 525 lesions, and the overall survival rates at 1, 2, 3, 4, and 5 years were 86%, 63%, 47%, 29%, and 24%, respectively. As they noted, these survival rates were substantially higher than those obtained with any chemotherapy regimen, and therefore, this provides indirect evidence that RFA therapy improves survival in patients with limited hepatic metastatic disease. Similar to RFA for hepatocellular carcinoma, RFA for colorectal metastases carries a relatively low major complication rate of approximately 5% (108,109).

One of the potential pitfalls of RFA is the "heat sink" effect in highly vascular tumors or tumors adjacent to large vascular structures, in which blood flow may permit cooling of the vascular portion of the tumor or the portion of the tumor that resides adjacent to the vessel. This heat sink phenomenon may prevent heating of the tumor to the point of tissue death and leave the viable tumor behind (99,110,111,112).

There are many other ablative approaches to liver tumors including microwave ablation, ultrasonic

ablation, and external beam irradiation. Which ablative therapy is best for localized nonresectable hepatic malignancies? At present there is no clear choice of the best

ablative approach for hepatocellular carcinoma (113), though a recent report suggests that the question is not which *single* therapy is best, but how to use combined local ablative therapies for the best outcome. Kitamoto et al. (114) described RFA without or with TACE. They found that combined RFA and TACE gave a markedly increased extent of induced coagulation compared with RFA alone. Although this was a small study, it highlighted the trend that many interventionalists are following, that is, using the ischemic/necrosing effects of TACE to enhance the ablative effects of RFA.

Transhepatic Portal Vein Embolization

Embolization of the portal vein branches of a portion of the liver leads to atrophy of the embolized territory with compensatory hypertrophy of the remaining liver. This technique has been used successfully before surgery for the development of the normal liver before hepatic resection in the treatment of liver tumors when there is doubt that the residual liver would otherwise be sufficient for performing adequate hepatic function (115,116).

There are four important considerations before performing PVE. First, the ratio of the future liver remnant (FLR) to the total estimated liver volume (TELV) should be calculated. Next, consideration should be given to differentiating cases with underlying diffuse liver disease from those without underlying liver disease because this influences the FLR needed to reduce postoperative morbidity and mortality. Although the absolute liver volume necessary after resection for adequate hepatic function has not been pinpointed, an FLR/TELV ratio of at least 25% is recommended in patients with otherwise normal livers, and a ratio of at least 40% is suggested in patients in whom the liver is considered compromised.

A third consideration is the presence of systemic disease, such as diabetes mellitus, that may limit hepatic hypertrophy. Finally, planning the type and extent of resection is important.

PVE using interventional techniques is typically performed by direct transhepatic puncture, entering one of the intrahepatic portal veins from either the left or the right sides (entering the side opposite to the portion of the liver that will be embolized). A vascular sheath is advanced into the portal venous system, and then, using angiographic techniques, selective portal venous imaging and embolization is performed. The latter technique is performed with PVA particles ranging from 300 to 700 μm and platinum microcoils when appropriate. Occasionally, larger PVA particles (up to 1,000 μm) are needed, and at some institutions, other embolic agents such as ethanol, thrombin, fibrin glue, microspheres, cyanoacrylate, ethiodized oil, and absorbable gelatin sponge have been used. The goal is to reduce flow in the target portal vein branches to stagnation or near-stagnation. At the completion of the embolization procedure, the sheath is removed by embolization of the transhepatic tract using microcoils or absorbable gelatin sponge.

Follow-up imaging is performed at 2 to 4 weeks because liver regeneration usually peaks within the first 2 weeks after PVE (117,118,119). Complications of PVE are uncommon but include risks of transhepatic puncture (e.g., intraperitoneal bleeding, hemophilia), portal vein thrombosis, and infection. Overall, a typical increase in the FLR/TELV ratio has been reported in the range of 8% to 12% in various series (120,121,122,123,124,125).

Percutaneous Abscess Drainage

Percutaneous drainage of liver abscesses has been an important component of medical care for many years. Pyogenic liver abscesses typically represent the final common presentation of many pathologic processes including infection that reaches the liver through the portal venous, hepatic arterial, or biliary tract invasion. The right hepatic lobe is involved in three fourths of the cases, and abscesses many be single or

multiple. Typically identified by ultrasound or CT scanning, both techniques are excellent methods for guiding catheter drainage procedures (126). Typically, catheters ranging form 8 to 14 Fr are used to drain pyogenic abscesses of the liver. Drainage volume is recorded regularly, and the tube is removed when little or no drainage persists. Catheter drainage is required for 5 to 7 days in most cases.

There are two controversial areas of percutaneous treatment of pyogenic liver abscesses. First, some believe that aspiration of the abscess along with intake of systemic antibiotics is sufficient for most patients, obviating the need for drainage catheter placement and management. Success rates between 58% and 88% that have been reported with this technique compare favorably with the reported success rates of 69% to 90% for catheter drainage (127,128,129,130,131,132,133). It should be noted, however, that repeat aspiration is required in more than half of the patients (134,135). Rajak et al. (136) found no difference in average time for clinical improvement, mean hospital stay, or average time for complete resolution of liver abscesses for catheter drainage compared with aspiration in a high-risk group of patients. This suggests that aspiration is a useful technique that should be considered, especially when catheter placement may be undesirable (e.g., in a patient with altered mental status who may dislodge the catheter).

The second controversial area is the use of antibiotics alone, without aspiration or drainage. Although this may suffice for patients with small abscesses and few other comorbid conditions, the indications for antibiotics alone have been not established. At the very least, aspiration can be used to obtain a specimen for microbiologic analysis and may suffice as a first-line treatment of pyogenic liver abscesses along with systemic antibiotics (126).

Amebic liver abscess is unusual in the United States but is a widespread problem globally (137). Medical treatment alone is recommended for patients suspected of having amebic liver abscess unless the patient is at high risk of abscess rupture. Risk factors include abscess cavity size greater than 5 cm, left hepatic lobe abscess, or failure to respond to medical therapy in 5 to 7 days (138,139). Typically, aspiration without drainage is recommended (137,140).

Percutaneous Cholecystostomy

The gallbladder may be drained with a catheter for various reasons, including cholecystitis, to gain access for the treatment of biliary stone disease, and for the drainage of the biliary system in the presence of common duct obstruction. Most cholecystostomy tubes are placed for drainage of an infected gallbladder, often in patients with contraindications to cholecystectomy. This is especially true for patients in the intensive care unit in whom acute cholecystitis may present without an obstructing stone (acalculous cholecystitis). For these patients, a cholecystostomy tube will permit effective drainage of the gallbladder while systemic antibiotics are administered. Often, the condition of acalculous cholecystitis will resolve with these measures alone, and the tube can be ultimately removed (141,142,143,144).

Drainage of the gallbladder has historically been accomplished by transhepatic puncture, with the initial passage of the needle through the liver and then into the gallbladder (144,145). Transhepatic gallbladder puncture was considered to be safer than direct gallbladder puncture, as it was commonly believed that there was less risk for bile leakage from the transhepatic approach because the liver would act to tamponade the puncture site during catheter drainage, as well as after catheter removal. Although the practice of transhepatic drainage of the gallbladder persists, it has been shown that a direct transperitoneal access can also been used safely (146), but there is no conclusive proof that a transhepatic route for drainage is necessary.

The procedure of catheter drainage of the gallbladder begins under sonographic or CT

guidance for puncture of the gallbladder, followed by fluoroscopy imaging to confirm that the drainage catheter has been inserted into an acceptable position. At the time of puncture, the bile is examined and sent to the laboratory for culture. An 8- to 14-Fr drainage catheter is inserted over the guide wire, with its tip in the gallbladder, usually using a catheter with a Cope loop design at its tip so that the catheter cannot be dislodged. Drainage volume from the cholecystostomy tube should be charted regularly.

When bile output from the cholecystostomy tube decreases, it could be due to one of three causes. First, the tube may have become intermittently obstructed. Second, the tube may have become dislodged from the gallbladder. Finally, the cystic duct may have opened, allowing the bile to pass down the common bile duct and into the duodenum rather than out through the catheter. The reason for the diminishing bile drainage should be evaluated fluoroscopically, injecting contrast through the cholecystostomy tube and into the gallbladder (if the tube has not become dislodged). If the cystic duct is open, the tube is usually capped for a trial period of internal drainage and ultimately removed. If the cystic duct is not open, external biliary drainage should be continued.

Removal of a cholecystostomy tube is done typically under fluoroscopic control by partially removing the catheter over a guide wire. Contrast is injected during this maneuver to ensure that there is no demonstrable communication between the gallbladder and the peritoneal space. If spillage is noted, the tube is reinserted and checked again in several days, and the procedure of tube removal is repeated with fluoroscopic assessment until there is no bile spillage.

For some patients, obstruction of the cystic duct will persist, typically because of an impacted stone or other fixed obstruction. For patients who cannot tolerate cholecystectomy, the tube may be needed as long-term therapy, with external drainage and regularly scheduled tube changes.

Conclusion

A variety of percutaneous procedures have been developed for the diagnosis and treatment of hepatobiliary disease. These procedures are under constant evolution, with some being incorporated into clinical practice while others have been rendered obsolete. It is admittedly a daunting task for most clinicians to find their way through this labyrinth of imaging and therapeutic options. Therefore, it is increasingly essential that members of the clinical team become familiar with their group of interventional radiologists who understand the risks, benefits, and use of imaging techniques and percutaneous procedures. By developing a close working relationship with their interventional radiology colleagues, the clinical team members will more readily find safe and effective strategies to provide care for patients with hepatobiliary disease.

Annotated References

Hepatobiliary Angiography

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Liver Biopsy

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Transarterial Chemoembolization of Hepatic Tumors

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Chapter 6

Endoscopic Retrograde Cholangiopancreatography

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Key Concepts

- Endoscopic retrograde cholangiopancreatography (ERCP) with therapeutic intervention (endoscopic sphincterotomy, stone extraction, or stent placement) can achieve biliary decompression in more than 95% of cases of biliary obstruction.
- Pancreatitis, the most common complication of ERCP, occurs in approximately 5% of cases, and the rate of this complication does not increase with therapeutic intervention.
- Endoscopic sphincterotomy permanently abolishes the pressure gradient across the sphincter of Oddi but is not associated with long-term biliary complications. Patients with cirrhosis who undergo endoscopic sphincterotomy have a higher-than-average risk of complications.
- In cholangitis, the treatment of choice is emergency ERCP with biliary decompression, which results in lower mortality than does surgical decompression.
- In acute biliary pancreatitis, ERCP is indicated in the care of patients with severe pancreatitis and in those with persistent abnormalities in liver enzyme levels. The low likelihood of recovering a stone from the common bile duct in cases of mild biliary pancreatitis or rapid improvement in liver enzyme abnormalities does not justify the use of routine ERCP.
- ERCP remains the standard of care for the diagnosis of primary sclerosing cholangitis (PSC). Endoscopic therapy for PSC strictures that includes dilatation and stent placement improves outcome in the subset of patients with a “dominant” extrahepatic stricture.
- ERCP is the procedure of choice for the diagnosis and management of biliary complications after cholecystectomy and liver transplantation. Bile leaks have high healing rates with short-term stenting, whereas biliary strictures require an aggressive regimen of endoscopic dilatation and stenting.

Since its inception in 1968 (1), endoscopic retrograde cholangiopancreatography (ERCP) has been an important diagnostic and therapeutic tool in the management of hepatobiliary disease. The advantages of this endoscopic approach to the hepatobiliary system compared with the more invasive percutaneous or open

surgical routes led to a rapid expansion in its applications, including stone extraction, dilatation and stenting of benign and malignant strictures, and management of postoperative complications. With the recent advances in noninvasive imaging, including magnetic resonance cholangiopancreatography (MRCP) and multidetector row computed tomography (MDCT), ERCP has now become mostly a therapeutic procedure.

Indications and Contraindications

Indications

The main indication for ERCP is the evaluation and treatment of suspected biliary obstruction. The broader

indications for the procedure include pancreatic disorders and the need for endoscopic therapy within the biliary or pancreatic ducts (Table 6.1).

Table 6.1. Indications for Endoscopic Retrograde Cholangiopancreatography

Biliary tract disease
Obstructive jaundice
Cholestasis
Cholangitis
Postoperative and traumatic biliary complications, such as bile leak, fistula, stricture
Evaluation of sphincter of Oddi dysfunction by means of manometry
Acquisition of bile for diagnosis of microlithiasis
Sphincterotomy for complications of gallstone disease when the gallbladder will be left in situ
Pancreatic disease
Acute biliary pancreatitis if severe or with persistent biliary obstruction
Suspected pancreatic cancer
Suspected chronic pancreatitis
Endoscopic therapy for known chronic pancreatitis
Evaluation of pancreatic trauma
Pancreatic duct roadmap before surgery

In the evaluation of cholestasis or jaundice, ERCP should be reserved for cases in which a high index of suspicion for obstruction exists. The distinction between obstructive and nonobstructive jaundice can usually be made on clinical grounds alone. Typically, obstructive jaundice leads to biliary dilatation on imaging (ultrasound, MDCT, or MRCP) and elevated liver function test results, particularly the alkaline phosphatase and total bilirubin levels. Instances in which obstruction may not be accompanied by ductal dilatation include obstruction of recent onset (because ductal diameter is partly related to the duration of obstruction) and primary sclerosing cholangitis (PSC). Once the diagnosis of obstructive jaundice is made, the appropriate timing of the ERCP must be determined. Acute cholangitis, characterized by fever, jaundice, and right upper quadrant abdominal pain, requires urgent biliary decompression by ERCP if it does not improve with conservative management. In the setting of biliary pancreatitis, published results

have shown a clear benefit of urgent ERCP (within 24 to 48 hours) in cases of severe biliary pancreatitis (2,3,4), but less so for mild pancreatitis, in which the likelihood of recovering a stone is lower and spontaneous recovery without complications higher.

Sphincter of Oddi dysfunction (SOD) is suspected when biliary or pancreatic symptoms occur or recur after cholecystectomy. SOD is classified into three types (5). The triad of elevation of serum liver enzyme levels, dilatation of the common bile duct (CBD), and poor drainage on a diagnostic cholangiogram is strongly suggestive of the diagnosis of type I biliary SOD, which is treated with empiric sphincterotomy. If only one or two of these three features are present (type II SOD), sphincter manometry is performed on both biliary and pancreatic sphincters. Normal basal sphincter pressure is less than 40 mm Hg, and phasic contractions are usually no higher than 250 mm Hg. Sphincterotomy is indicated and successful when the diagnosis of SOD is accurate. Type III SOD (pain syndrome) has none of the aforementioned features and is usually managed conservatively but sphincter of Oddi manometry may be helpful in proving normalcy.

The growth of laparoscopic cholecystectomy and liver transplantation has led to an increase in the incidence of postoperative complications in the biliary tract. Biliary-type pain, elevated cholestatic liver enzyme levels, or abnormal imaging in the postoperative setting usually constitutes an indication for ERCP.

The role of ERCP in the evaluation of chronic abdominal pain that has defied diagnosis with less invasive testing is questionable and carries the highest risk of post-ERCP complications, especially pancreatitis. The development of MRCP has provided a reliable and safe means of imaging the biliary and pancreatic ductal systems and has therefore lowered the utility of ERCP as a purely diagnostic modality.

Contraindications

The same general contraindications that apply to other endoscopic procedures also apply to ERCP. Refusal by the patient to undergo the procedure or an acute cardiovascular or pulmonary condition are the only true contraindications. A Zenker's diverticulum, esophageal diverticulum, or tight esophageal stricture can complicate passage of a side-viewing duodenoscope. Previous surgery on the upper gastrointestinal (GI) tract (e.g., Billroth II partial gastrectomy) is not a contraindication, although the altered anatomy may render the procedure technically more difficult.

ERCP is not contraindicated during pregnancy, but should be performed judiciously (6,7). With a pregnant patient, radiation exposure is minimized by placement of a lead shield to protect the patient's abdomen, scant use of fluoroscopy, and avoidance of spot radiographs.

History of an adverse reaction to an ionic contrast medium is not a contraindication to ERCP. Systemic absorption of contrast medium injected into the biliary or pancreatic ducts is negligible, and reports of adverse reactions at the time of ERCP are rare (8). In the care of patients with a documented history of a severe reaction (laryngeal edema, anaphylaxis, or vascular collapse), it is our policy to administer glucocorticoids and antihistamines, starting 12 hours before the procedure.

Techniques

Preparation

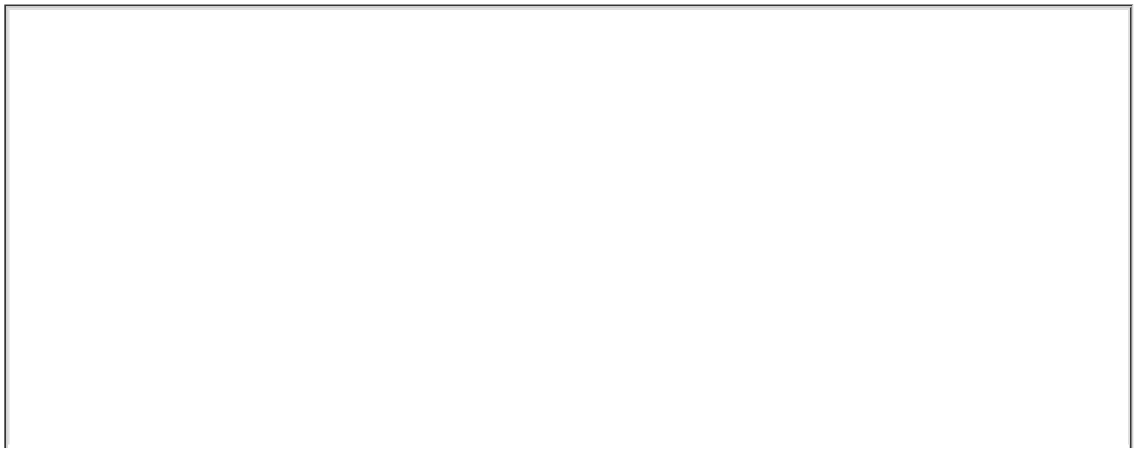
After a detailed discussion about informed consent and addressing the patient's concerns, he or she is placed in the prone “swimmer's” position. A combination of intravenous midazolam and fentanyl provides conscious sedation. The patient is given supplemental oxygen and is monitored throughout the procedure for heart rate, blood pressure, and pulse oximetry. Capnography monitoring is likely to become standard in the near future. Propofol (monitored anesthesia care) and general anesthesia have been used increasingly for ERCP in some centers.

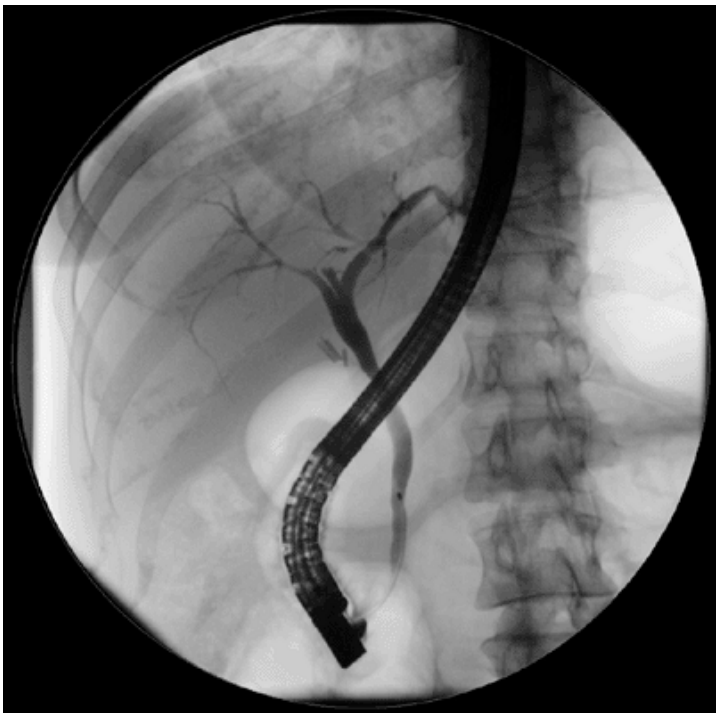
Prophylactic antibiotics are administered according to the guidelines of the American Society for Gastrointestinal Endoscopy (ASGE) (9), although their value has been questioned. If injected contrast does not subsequently drain adequately (because of a stricture or obstruction) and adequate endoscopic drainage cannot be provided, intravenous ampicillin (2 g) and gentamicin (1.5 mg/kg) should be given empirically immediately after the procedure. In the setting of PSC, periprocedural antibiotics are universally recommended because of the propensity of these patients to develop post-ERCP cholangitis.

A “therapeutic” side-viewing duodenoscope is now almost universally used in adults as the standard instrument. It is approximately 13 mm in diameter and contains a 4.2-mm diameter accessory channel. It is carefully passed through the upper GI tract and into the duodenum. Excessive duodenal motility can be suppressed by intravenous administration of 0.2 to 0.5 mg of glucagon. At this time, cannulation of the CBD is attempted using a catheter or sphincterotome. The CBD and ventral pancreatic duct share the papillary orifice in more than 90% of cases. Selective cannulation of the bile duct requires a detailed knowledge of the local anatomy and a range of technical maneuvers that require prolonged training to acquire.

The Normal Cholangiogram

Bile duct cannulation allows the delineation of the biliary tree anatomy, including intrahepatic and extrahepatic ducts, as well as the cystic duct and gallbladder (Fig. 6.1). The muscular wall of the distal CBD can cause smooth tapering or a shelf-like “notch” that may be mistaken for a stricture. A contracted sphincter also can simulate an impacted stone (pseudocalculus sign). Care must be taken to identify the cystic duct because its origin in the CBD is quite variable, which can make the identification of the common hepatic duct and confluence of the hepatic ducts difficult.





▪ **Figure 6.1** Normal postcholecystectomy endoscopic retrograde cholangiogram.

The left and right hepatic ducts fuse 7 to 15 mm below the hilum at the confluence. The left hepatic duct is slightly longer than the right hepatic duct. With the patient in the usual prone position during ERCP, the left hepatic duct is in a dependent position and therefore fills preferentially when contrast material is injected into the CBD. Maneuvers for filling the right hepatic ductal system include tilting the head of the table down 15 to 20 degrees, injecting directly into the right system or through an inflated balloon in the common hepatic duct (occlusion cholangiography), or turning the patient to the supine position.

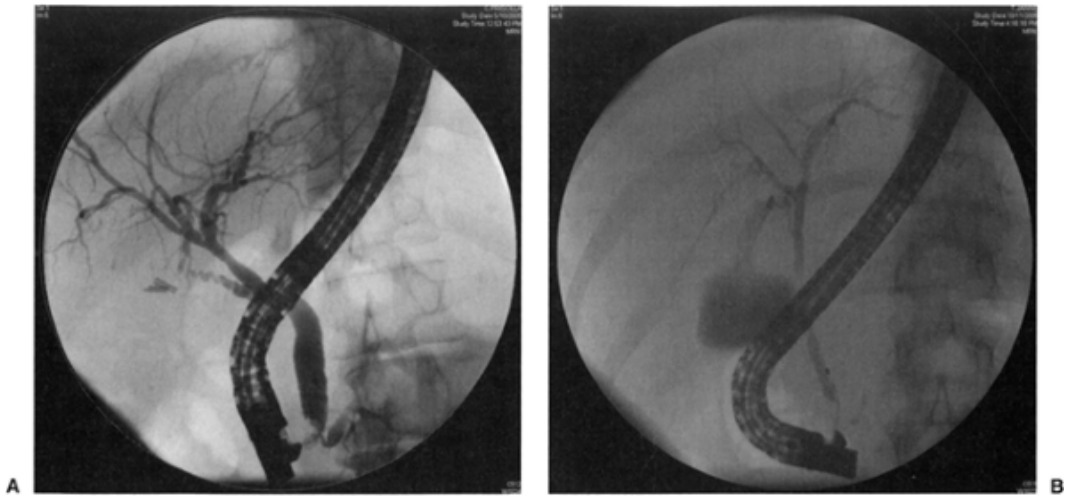
There are many variations in biliary tract anatomy. In approximately 2% of cases, the cystic duct drains into the confluence, so there is no true common hepatic duct. In 1% of the population, the right hepatic duct, or a second abberrant right hepatic duct, drains directly into the gallbladder or the cystic duct. This variant may lead to postoperative complications if the presence of this configuration is not recognized and the aberrant duct is inadvertently clipped or injured during laparoscopic cholecystectomy (Fig. 6.2).

Therapeutic Endoscopic Retrograde Cholangiopancreatography

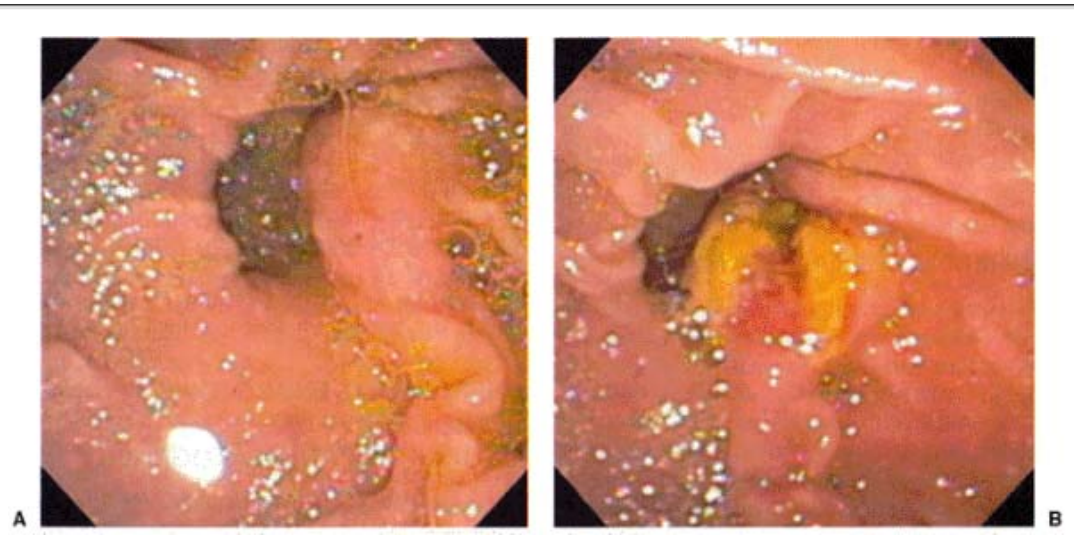
The most important therapeutic ERCP technique and often the first step in a therapeutic procedure is

endoscopic sphincterotomy (ES). A sphincterotome, a plastic catheter with an exposed metal wire at the distal end, is used in the procedure. To perform an ES, the tip of the sphincterotome is bowed to isolate the wire, and is positioned such that only a portion of the bowed wire is in contact with the papilla. A cutting

current is applied in the direction of the bile duct (Fig. 6.3). After ES, the stones can be extracted through the papilla using balloon catheters or Dormia-type baskets (Fig. 6.4A, B). Failure to clear the duct may be due to the shape, number, or size of stones (stones >15 mm being considered large) or to a distal obstruction, such as mass or stricture. These difficult bile duct stones can be extracted in most cases after mechanical lithotripsy (Fig. 6.4E, F, G). This procedure is performed with a specialized basket that is cranked closed against an unyielding metal sheath, fragmenting the stone within the bile duct. Stones refractory to mechanical lithotripsy can be fractured using electric hydraulic lithotripsy (EHL) (see the subsequent text), laser lithotripsy (10), or extracorporeal shock wave lithotripsy (11). Dissolution therapy with solvents has not gained wide acceptance in the United States.



▪ **Figure 6.2 A:** Normal intrahepatic ductal anatomy showing the typical "trifurcation" at the confluence of the right and left hepatic ducts. **B:** Aberrant right hepatic ductal system with a second duct arising close the cystic duct.



▪ **Figure 6.3** Endoscopic views of papilla **(A)** before sphincterotomy and **(B)** after sphincterotomy.

Biliary obstruction can also be decompressed using either plastic biliary stents or self-expanding metal

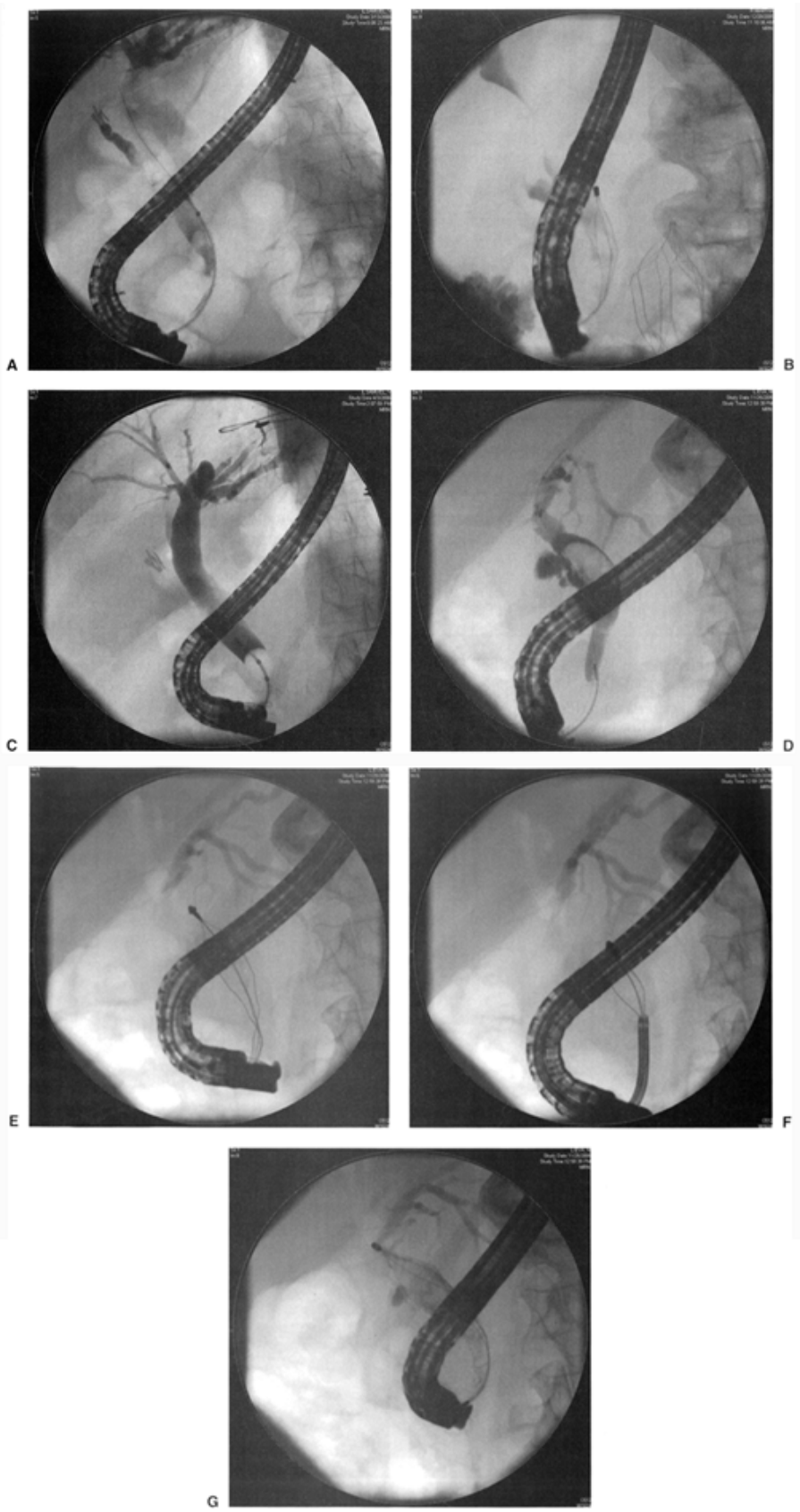
stents (SEMS), which are placed over a guidewire (Fig. 6.5A, B). When using biliary stents, 10-Fr diameter stents should be placed whenever possible because narrower stents have been shown to have diminished patency rates in clinical trials (mean patency, 144 days for the 10-Fr stent vs. 67 days for the 8-Fr stent) (12). Biliary stents are usually replaced in 2 to 3 months. Metallic stents are reserved for palliation of malignant obstruction or as a last resort in difficult benign strictures.

Tissue sampling from the bile duct can be accomplished using cytology brushes (with or without a guidewire) or small biopsy forceps. Biliary dilatation can be performed with dilating balloons or catheters of gradually increasing diameter, both of which can be passed over a guidewire.

Cholangioscopy

Direct endoscopic visualization of the bile ducts has become possible using thin-caliber cholangioscopes of 3.3 mm diameter or less. The “baby” cholangioscope is passed through the “mother” endoscope and into the bile duct with or without the assistance of a guidewire. The “baby” endoscope has only two-dimensional tip movement and is usually controlled by a second endoscopist. Baby endoscopes have a small accessory channel that allows flushing of the bile duct and passage of a biopsy forceps or other small-caliber device. One of the important applications of cholangioscopy is EHL, which successfully fragments large stones that cannot be extracted using mechanical lithotripsy (13,14). This technique requires direct apposition of a thin electrode

probe against the stone. Electric sparks are delivered in short pulses within a liquid medium, leading to the generation of pressure waves. Care must be taken to avoid injuring the bile duct.



▪ **Figure 6.4** Endoscopic retrograde cholangiopancreatography showing **(A)** a stone in the distal bile duct, **(B)** basket extraction, **(C)** balloon extraction,

(D) a large stone in the bile duct and the sphincterotome positioned for sphincterotomy, **(E)** lithotripsy basket entrapping the stone, **(F)** mechanical lithotripsy crushing the stone, **(G)** basket removal of fragments.

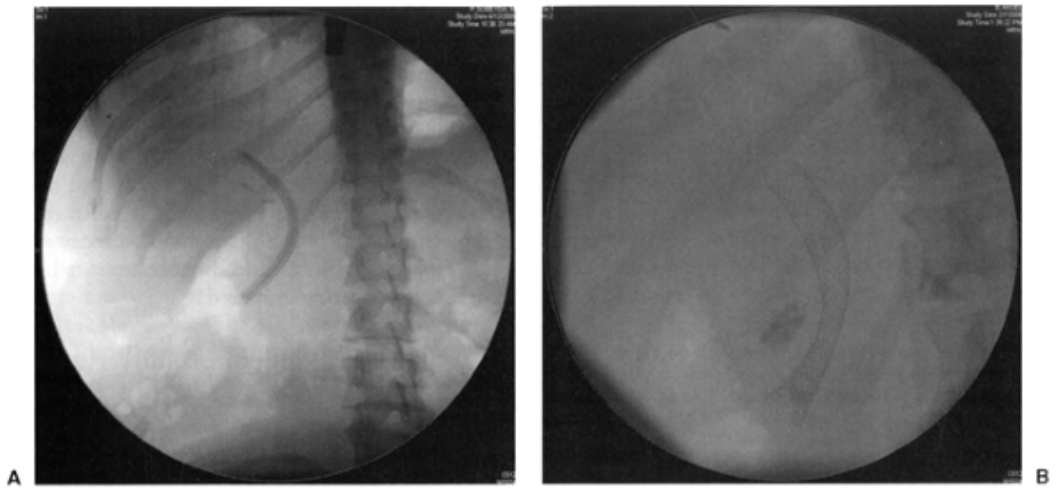
Complications

A consensus conference in 1991 (15) classified ERCP-related complications as mild, moderate, or severe on the basis of clinical criteria, and this grading system has been used in most studies involving ERCP ever since (Table 6.2).

In a study of more than 2,000 ERCPs with ES (16), the mortality rate related to the procedure was 0.4%. The overall complication rate was 9.8%, and severe complications occurred in 1.6% of cases (Table 6.3). The most common ERCP-specific complication was pancreatitis, occurring in 5.4% of patients undergoing sphincterotomy and accounting for more than one half of all cases of complications (13). Only 9 of the 127 (7%) cases of pancreatitis were graded as severe.

Post-ERCP pancreatitis is characterized by abdominal pain and elevated levels of pancreatic enzymes. Predisposing risk factors for pancreatitis include female sex and a normal serum bilirubin level (17), repeated attempts at bile duct cannulation, and “precut” or access papillotomy with a needle knife (16). The incidence of pancreatitis does not seem to differ between purely diagnostic and therapeutic ERCP (17). However, the risk of ERCP-induced pancreatitis in the setting of SOD is increased and has been reported to be as

high as 20% (18). Intravenous infusions of somatostatin, an inhibitor of pancreatic enzyme secretion, and gabexate, a synthetic protease inhibitor (19), and oral allopurinol (20,21) may be effective in the prevention of pancreatitis. Other prophylactic agents are under evaluation.



▪ **Figure 6.5 A:** Two plastic stents in position in bile duct, **(B)** self-expanding metallic stent in bile duct.

Bleeding from the sphincterotomy site is the second most common ERCP-specific complication, occurring in up to 2% of cases (16). Most episodes are evident at the time of sphincterotomy, although delayed hemorrhage can occur up to 2 weeks after ES. Most cases of bleeding can be managed endoscopically with local epinephrine injection, multipolar coagulation, or hemostatic clips. Angiographic embolization is rarely required for severe bleeding associated with the retroduodenal artery, which is in the vicinity of the sphincterotomy site in 4% of patients.

Table 6.2. Grading System for the Major Complications of Endoscopic Retrograde Cholangiopancreatography and Endoscopic Sphincterotomy

Complication	Mild	Moderate	Severe
Bleeding	Clinical (not just endoscopic) evidence of bleeding, hemoglobin drop by <3 g/dL, and no need for transfusion	Transfusion (4 U or less), no angiographic intervention or surgery	Transfusion 5 U or more or angiographic or surgical intervention
Perforation	Possible, or only very slight leak of fluid or contrast	Any definite perforation managed medically for	Medical treatment for >10 d or percutaneous or

	medium, managed with fluids and suction for 3 d or less	4–10 d	surgical intervention
Pancreatitis	Clinical pancreatitis, amylase level at least three times the normal value for >24 h after the procedure, necessitating admission or prolongation of planned admission to 2–3 d	Pancreatitis requiring hospitalization for 4–10 d	Hospitalization for >10 d, hemorrhagic pancreatitis, phlegmon, pseudocyst, or intervention (percutaneous drainage or surgery)
Infection (cholangitis)	>38°C 24–48 h	Febrile or septic illness necessitating >3 d of hospital treatment or endoscopic or percutaneous intervention	Septic shock or surgery
Basket impaction	Basket released spontaneously or by repeated endoscopy	Percutaneous intervention	Surgery
Any intensive care unit admission after a procedure grades the complication as severe. Other rarer complications can be graded by the length of needed hospitalization.			

ES in the setting of cirrhosis carries an increased risk of bleeding, and should therefore be performed judiciously. Multivariate analysis has revealed that

coagulopathy, rather than cirrhosis per se, was the risk factor associated with a threefold increased risk of bleeding (16). Even so, the adjusted odds ratio for all ES complications in patients with cirrhosis was 2.9 (95% confidence interval, 1.5 to 5.9). This high overall rate of complications of ES among patients with

cirrhosis was also demonstrated in a retrospective study conducted in France on a series of 52 patients with cirrhosis (18, Child-Pugh class A; 22, class B; 12, class C) (22). Four of the 52 patients in that series (7.7%), all Child-Pugh class C, died within 5 days of the procedure because of bleeding, perforation, or sepsis. The 1-month mortality rate was 12.5%.

Table 6.3. Complications of Endoscopic Sphincterotomy

Type of complication	Percentage with complication	Percentage with severe complication
Pancreatitis	5.4	0.4
Hemorrhage	2.0	0.5
Perforation	0.3	0.2
Cholangitis	1.0	0.1
Cholecystitis	0.5	0.1
Miscellaneous[a]	1.1	0.3
Any complication	9.8	1.6

[a]Includes cardiopulmonary complications, ductal perforation by guidewire, stent malfunction, antibiotic-induced diarrhea, indeterminate fluid collection, and infection of pancreatic pseudocyst.
Adapted from Freeman ML, Nelson DB, Sherman S, et al. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996;335:909–918, with permission.

If ES is performed on a patient with cirrhosis, admission for observation is prudent. It is recommended that patients with cirrhosis who undergo ES receive preprocedural transfusions of platelets and fresh frozen plasma for correction of coagulopathy and that they receive periprocedural antibiotics.

An alternative to ES in this setting is balloon dilatation of the sphincter, which has shown equal efficacy in CBD clearance and fewer bleeding episodes than ES in patients without cirrhosis (23), but it may be associated with more postprocedural pancreatitis (24). A recent report from Korea showed that sphincter dilatation caused fewer bleeding episodes than did ES in patients with cirrhosis and coagulopathy (25). A recent meta-analysis concluded that balloon dilatation may be the preferred method in patients with an underlying coagulopathy (26).

Perforations are rare but can occur when the sphincterotomy incision extends

beyond the intramural segment of the bile duct into the retroperitoneal space (27). Perforations are usually identified during the procedure but may present afterward with abdominal pain, with or without abdominal distension. The diagnosis is made by the presence of retroperitoneal air on computed tomography (CT) scan, and most can be managed conservatively with antibiotics and nasogastric suction.

Cholangitis occurs as a complication in 1% to 3% of ERCP procedures and usually results from inadequate ductal drainage. Bile should be aspirated before injection of contrast medium into an obstructed biliary system, and the least amount of contrast medium necessary to define the lesion should be injected. If adequate drainage of contrast cannot be accomplished endoscopically, a percutaneous transhepatic drainage procedure is urgently indicated.

Benign or Premalignant Biliary Obstruction

Choledocholithiasis

The annual incidence of gallstone disease in patients with cirrhosis appears to be 2.6% to 5.5%, which is higher than that among the population without cirrhosis. (28). In patients with liver disease and suspected choledocholithiasis, the decision to proceed to ERCP should be made carefully. Trends in liver enzymes in the first 48 to 72 hours after the initial presentation are an important indicator of the likelihood of recovering a stone during ERCP (29). When the results of the liver function tests normalize, the likelihood of finding choledocholithiasis is lower (14%) than that among patients whose liver function test results remain unchanged or increase (90% likelihood of finding a CBD stone by ERCP). Improvement in liver function test results that falls short of normal values is associated with an intermediate prevalence of stones. Imaging studies can help weigh the need for ERCP. Studies have shown that a combination of elevated liver function results and abnormal radiographic findings (e.g., dilated CBD) are more predictive of CBD stones than is either sign alone (30). Transabdominal ultrasonography has high diagnostic accuracy for intrahepatic calculi but depicts only two thirds of CBD stones. MRCP has a sensitivity of greater than 90%, but its accuracy is directly correlated with the size of the stone (31,32,33). Either transabdominal ultrasonography or MRCP is a reasonable first test when choledocholithiasis is suspected because these modalities have a sensitivity greater than 90%. MDCT and virtual cholangiography may be a third option (34). Endoscopic ultrasound has also been shown to be helpful in diagnosing CBD stones (35) but is not yet a primary option. If there is evidence of a stone or abnormal biliary dilatation, then ERCP should be performed. A meta-analysis addressing the subject of predictive factors showed that cholangitis, CBD stones found by ultrasound examination, and preoperative jaundice were the three

strongest predictive indicators of the presence of CBD stones (36).

The role of ERCP in choledocholithiasis is to confirm the diagnosis and extract stones therapeutically, as described earlier. Accomplishing timely biliary drainage is imperative in the setting of cholangitis. If a difficult stone resists attempts at extraction or fragmentation, a plastic stent should be placed across the stone and extraction should be attempted at a later date. Biliary stents have been successful at partial stone disintegration over several weeks to months, likely by mechanical abrasion of the stone. Stone extraction is often easier at subsequent attempts (37). The duct should ultimately be cleared of stones because long-term

indwelling plastic stents are associated with a risk of cholangitis of up to 40% (38).

Before the use of laparoscopic cholecystectomy, many patients with symptomatic choledocholithiasis with minimal or no symptoms attributable to the gallbladder, or those considered poor surgical candidates for open cholecystectomy, were treated with ES alone, and their gallbladders were left in situ. For cholecystitis and choledocholithiaisis in patients with cirrhosis, the high operative morbidity and mortality of cholecystectomy mitigate in favor of endoscopic management of biliary tract disease whenever possible. As noted previously, patients with cirrhosis also have a higher than average risk of bleeding after ES; however, this risk may not normalize even when coagulopathy is corrected before the procedure (39). Long-term follow-up study with high-risk patients showed minimal development of gallbladder complications, and only a few patients needed cholecystectomy (40). There is now growing evidence that elderly patients or those at high operative risk for undergoing laparoscopic cholecystectomy can be treated similarly with ES and observation, with cholecystectomy being reserved for patients who have recurrent biliary symptoms. Advanced cirrhosis with cholecystitis may also be successfully managed with the placement of a long plastic stent from the gallbladder to the duodenum (41,42), which serves as a bridge to liver transplantation.

Sclerosing Cholangitis

PSC is a chronic cholestatic disorder characterized by fibrosing inflammation of the intrahepatic and extrahepatic biliary tree that progressively leads to cirrhosis, portal hypertension, liver failure, and death within a mean of 12 years after diagnosis. There is a strong association with inflammatory bowel disease. ERCP is the standard for diagnosis of PSC, and the availability of this modality has led to the diagnosis of an increased number of cases in the asymptomatic, preicteric phase. Recently, MRCP has been shown to be as accurate as and more cost-effective than ERCP (43,44,45) and may eventually be the diagnostic study of choice.

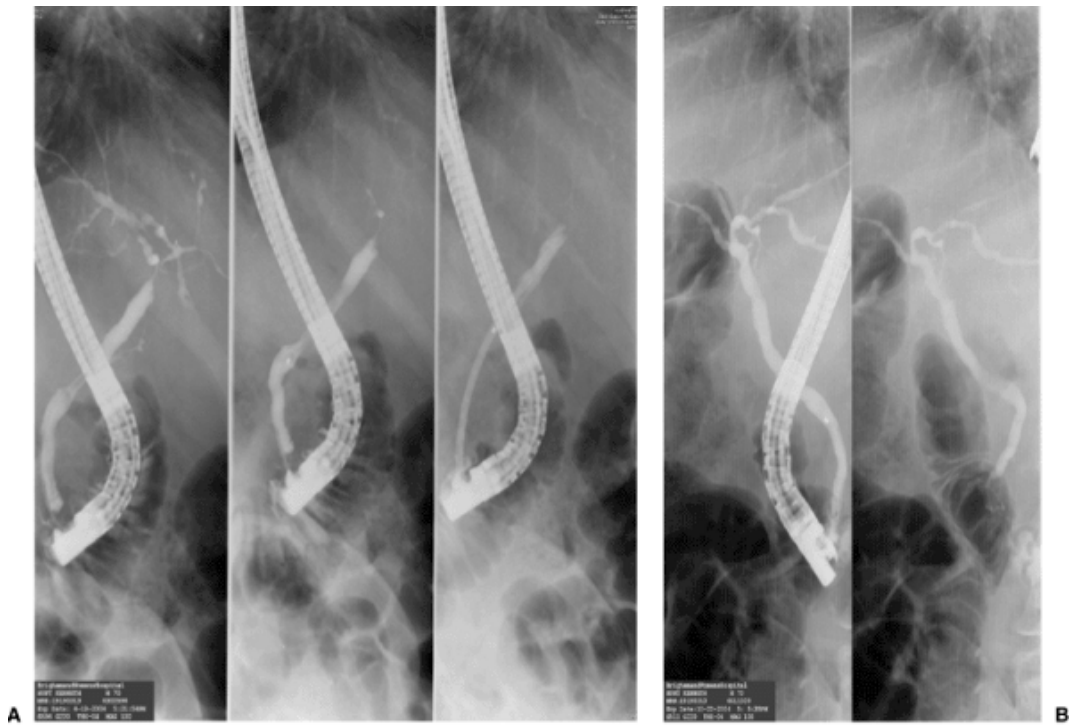
The cholangiogram in PSC shows characteristic diffuse, multifocal strictures of the intrahepatic and extrahepatic ducts, with areas of ectasia that give a beaded appearance (Fig. 6.6A). A rare variant termed *small duct sclerosing cholangitis* involves ducts of such small caliber that cholangiographic findings appear normal. Biliary strictures may prevent opacification of the full ductal system, which can be overcome by occlusion cholangiography with an inflated balloon catheter above the cystic duct entry. However, the same strictures often prevent complete drainage of injected contrast medium, resulting in an increased incidence of post-ERCP cholangitis if antibiotics are not used. Unfortunately, the routine use of antibiotics before and after the procedure does not prevent cholangitis in all cases. Therefore, given the possible complications of obtaining a cholangiogram, ERCP should be performed judiciously on patients with known PSC.

One of the most important and difficult differentiations to be made during cholangiography is that between benign strictures of PSC and cholangiocarcinoma, which arises as a complication of PSC in up to 15% of cases. The onset of weight loss or sudden deterioration of jaundice or pruritus may herald the onset of cholangiocarcinoma and warrants investigation using cholangiography. Brush cytologic examination of strictures at the time of ERCP has a sensitivity of 60% for detection of cholangiocarcinoma (46). The combination of brush cytology, tissue deoxyribonucleic acid analysis, and serum

CA 19-9 and carcinoembryonic antigen level determination was reported to increase the sensitivity and specificity in diagnosing cholangiocarcinoma in patients with PSC (47). To date, routine surveillance of patients with PSC for the development of cholangiocarcinoma has not been recommended.

A number of other conditions can mimic the cholangiographic appearance of PSC (Table 6.4). Biliary tract involvement of autoimmune pancreatitis has been described (48). Cirrhosis of any cause can lead to blunting and thinning of the intrahepatic tree, but intervening dilatation is not usually present. Multifocal liver metastases, lymphoma, and polycystic liver disease can similarly cause multifocal intrahepatic narrowing, and nodal involvement at the porta hepatis can mimic extrahepatic involvement. Acquired immunodeficiency syndrome (AIDS) cholangiopathy associated with opportunistic infection is discussed later (see “Acquired Immunodeficiency Syndrome Cholangiopathy”). Postoperative biliary strictures, which have an ischemic pathophysiologic mechanism, can lead to secondary biliary cirrhosis and have an appearance similar

to that of PSC, but the extrahepatic bile duct distal to the injury is usually normal.



▪ **Figure 6.6 A:** Sclerosing cholangitis affecting the extrahepatic and intrahepatic bile ducts. **B:** The same patient after medical and endoscopic therapy, showing improvement.

The benefit of endoscopic therapy for strictures in PSC is controversial. The rationale for endoscopic therapy is that back-pressure from high-grade strictures is at least in part responsible for the progression of liver disease. The ideal candidate for endoscopic therapy, therefore, has a discrete high-grade extrahepatic obstruction with limited intrahepatic involvement (Fig. 6.3B).

Unfortunately, this sort of “dominant” stricture is seen in only 20% of patients with PSC. The study by a group from Amsterdam has reported significant benefits from short-term endoscopic treatment of 32 patients with a dominant stricture (49). Short-term stenting with a 7- or 10-Fr stent after stricture dilatation resulted in significant improvement in mean scores for pruritus, fatigue, and right upper quadrant pain 2 months after the procedure. Jaundice resolved in 12 of 14 patients. There was evidence that the beneficial effect was durable because no reintervention was needed by 80% and 60% of patients 1 and 3 years after the procedure, respectively. Another group has described a slightly different approach in which balloon dilatation was the primary therapy, repeated yearly or more often if clinical or biochemical markers worsened (50). Stent placement was performed for 4 to 12 weeks only if balloon dilatation was deemed unsatisfactory. A group of 63 patients was therefore observed for a median of 34 months and needed a mean of 2.3 dilatations (range, 0 to 7) and a mean of 2.2 stents (range, 0 to 5). Compared with the predicted survival rate calculated using the Mayo Clinic survival model, this aggressive approach resulted in a significant increase in 1-, 3-, and 5-year survival rates from 92%, 77%, and 65% to 97%, 87%, and 83%, respectively (P = 0.03). Although this study was conducted with historical controls and the results may have been influenced by the concomitant use of ursodiol in the care of all the patients in the study, the results emphasized the potential impact of endoscopic therapy in altering the course of PSC. However, these therapies, even when combined, may not always be successful (51). An analysis of published papers revealed that the predictors of positive clinical and laboratory success include the presence of a dominant stricture, endoscopic therapy, and high serum bilirubin levels (52).

Hemobilia

Hemobilia refers to bleeding into the biliary tree. Today, the most common causes of hemobilia are endoscopic,

surgical, or radiologic procedures directed at the liver and biliary tract. The incidence of hemobilia following percutaneous liver biopsy is between 0.06% and 1% (53). Other causes of hemobilia include blunt or penetrating liver trauma, hepatic or biliary tumors, ascariasis, hydatid disease of the liver, and vascular fistulas. Hemobilia manifests as the classic Sandblom triad of upper GI bleeding, right upper quadrant pain, and jaundice in only 22% of patients (53). If bleeding is brisk, the presentation may be dominated by signs of hemorrhage, which can be severe and sometimes fatal. Slower rates of bleeding lead to clot formation within the bile ducts and can cause pancreatitis, cholecystitis, or cholangitis. If the pressure within the bleeding vessel is low, as in a hepatic vein or portal vein, the flow may be reversed and bile may enter the bloodstream, a rare condition called *bilhemia.*

Table 6.4. Conditions that can Mimic Primary Sclerosing Cholangitis During Cholangiography

Neoplastic disease
Cholangiocarcinoma
Liver metastasis
Porta hepatis lymph node metastasis

Lymphoma
Cirrhosis
Polycystic liver disease
Infectious disease
Ascending cholangitis
Intrahepatic stones with bacterial infection
Multiple hepatic abscesses
AIDS cholangiopathy (cytomegalovirus, *Cryptosporidium*)
Graft versus host disease
Hepatic arterial ischemia
Posttraumatic
Postsurgical, including liver transplantation
Chronic rejection after liver transplantation
Biliary involvement by autoimmune pancreatitis

AIDS, acquired immunodeficiency syndrome.

Ultrasonographic findings can be misleading in hemobilia because blood in the bile duct can be isoechoic to liver parenchyma, so the bile ducts may not be visualized at all. On ERCP, fresh blood or clot often extrudes from the papilla. Cholangiography demonstrates intraductal clots that can appear string-like, if they have formed a cast of the bile duct, or rounded, mimicking bile duct stones. Balloon extraction of the clot during ERCP is an effective means of relieving bile duct obstruction (54). When the bleeding site is in the distal CBD, endoscopic coagulation, injection of diluted epinephrine, or clip application usually achieves hemostasis. However, in most cases of severe hemobilia, bleeding arises from an intrahepatic arteriobiliary fistula that is not amenable to endoscopic therapy. These cases necessitate hepatic arteriography and embolization.

Hepatobiliary Surgery and Complications

Cholecystectomy

Biliary leaks appear to be more common after laparoscopic cholecystectomy than after open cholecystectomy, ranging in incidence from 0.65% to 5% (55,56). Most bile duct leaks after laparoscopic cholecystectomy are from the cystic duct stump, although leakage can occur anywhere in the extrahepatic biliary tract, including the “ducts of Luschka” in the gallbladder bed (Fig. 6.7).

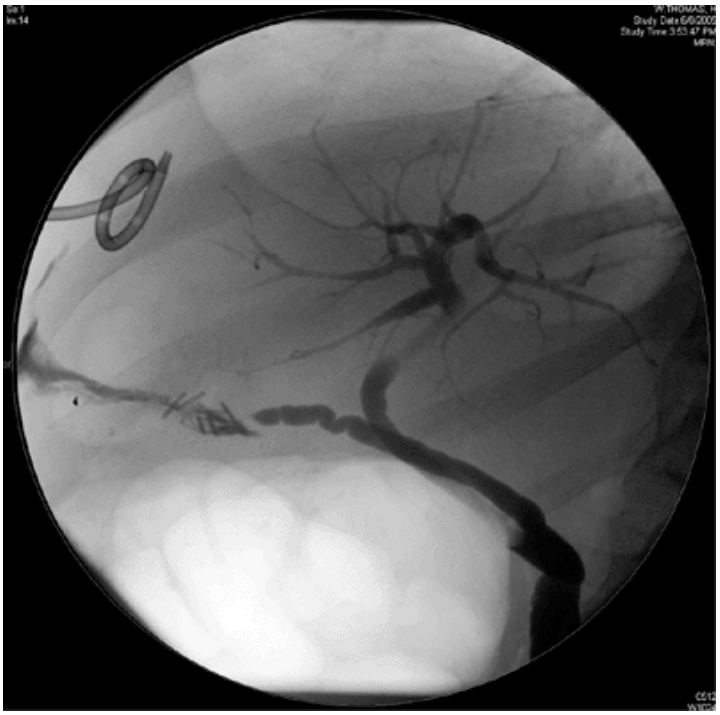
Symptoms of abdominal pain, tenderness, and fever can occur in the immediately postoperative period but are often not recognized until an average of 5 days after surgery. The diagnosis may be made by transabdominal ultrasonography, CT scan, or nuclear scintigraphy (57).

ERCP has three roles in the management of bile leaks. First, ERCP best demonstrates the anatomy and identifies and locates a leak. Second, it helps rule out any potential distal biliary obstruction, such as CBD stones (Fig. 6.8A), which could contribute to the development and persistence of a bile leak. Third, ERCP provides therapeutic options.

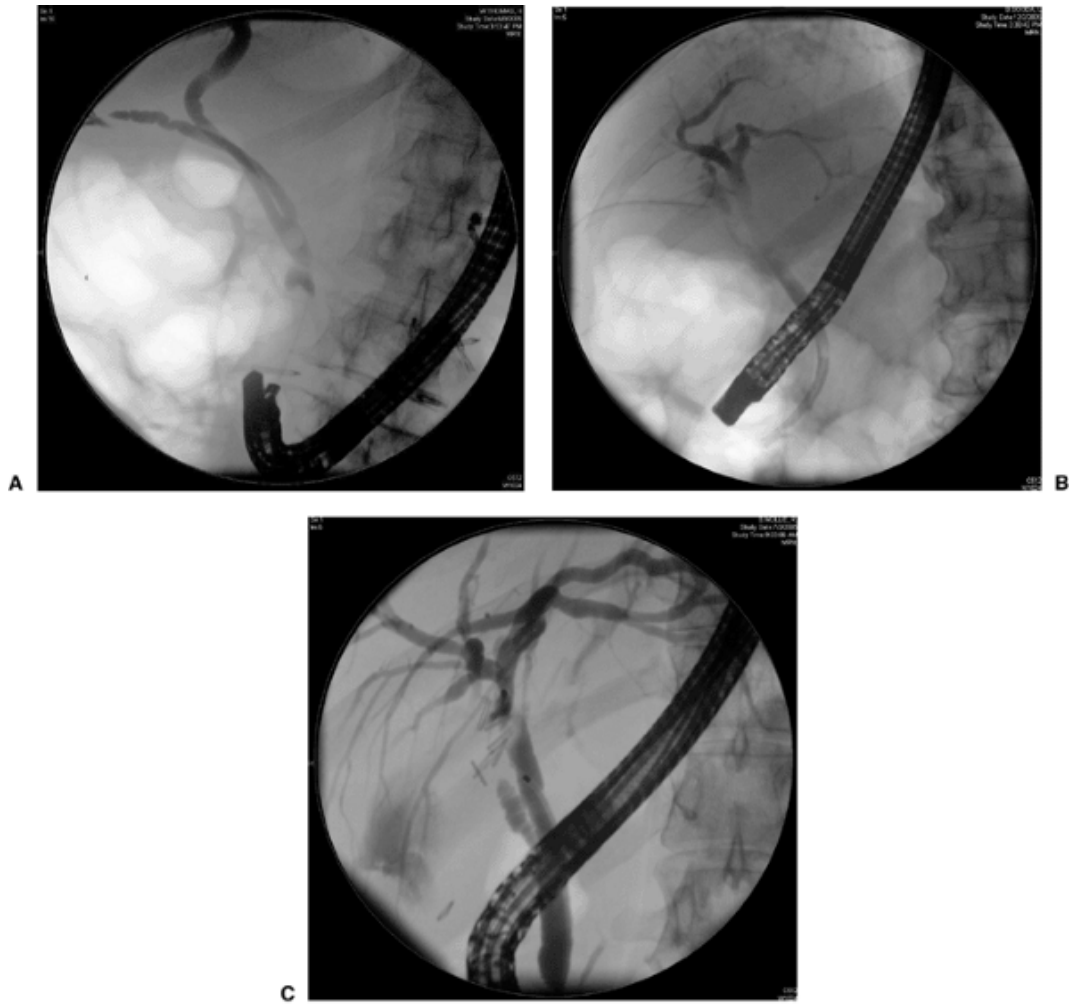
Although spontaneous closure of a leak has been described after percutaneous

drainage of the bile collection, most patients need intervention. ERCP is the therapy of choice for bile leaks because it is highly successful and carries less morbidity than does surgical repair. Endoscopic therapy has been shown to achieve closure in more than 90% of patients. When no distal biliary obstruction is found, the least invasive method of treatment is the placement of a 10-Fr stent without sphincterotomy. Placement of a stent across

the duodenal papilla is adequate treatment (58,59) (Fig. 6.8B). More complex bile leaks involving major injury to the CBD, common hepatic duct, or right hepatic duct necessitate stent placement across the injury site if possible for effective resolution and for reduction of stricture formation that may occur later (Fig. 6.8C). Nasobiliary tubes have also been shown to be successful (60). No data exist on the ideal period for stent placement, but 1 to 4 weeks is recommended in most series. At the time of stent removal, repeat cholangiography is not necessary if the patient is clinically well. Surgery may be necessary if, rarely, endoscopic management fails. Endoscopists must be aware of excluded hepatic duct segments that may leak but are disconnected from the biliary tract, as shown by ERCP. A knowledge of segmental intrahepatic ductal anatomy is essential.



▪ **Figure 6.7** Cystic duct leak after laparoscopic cholecystectomy.



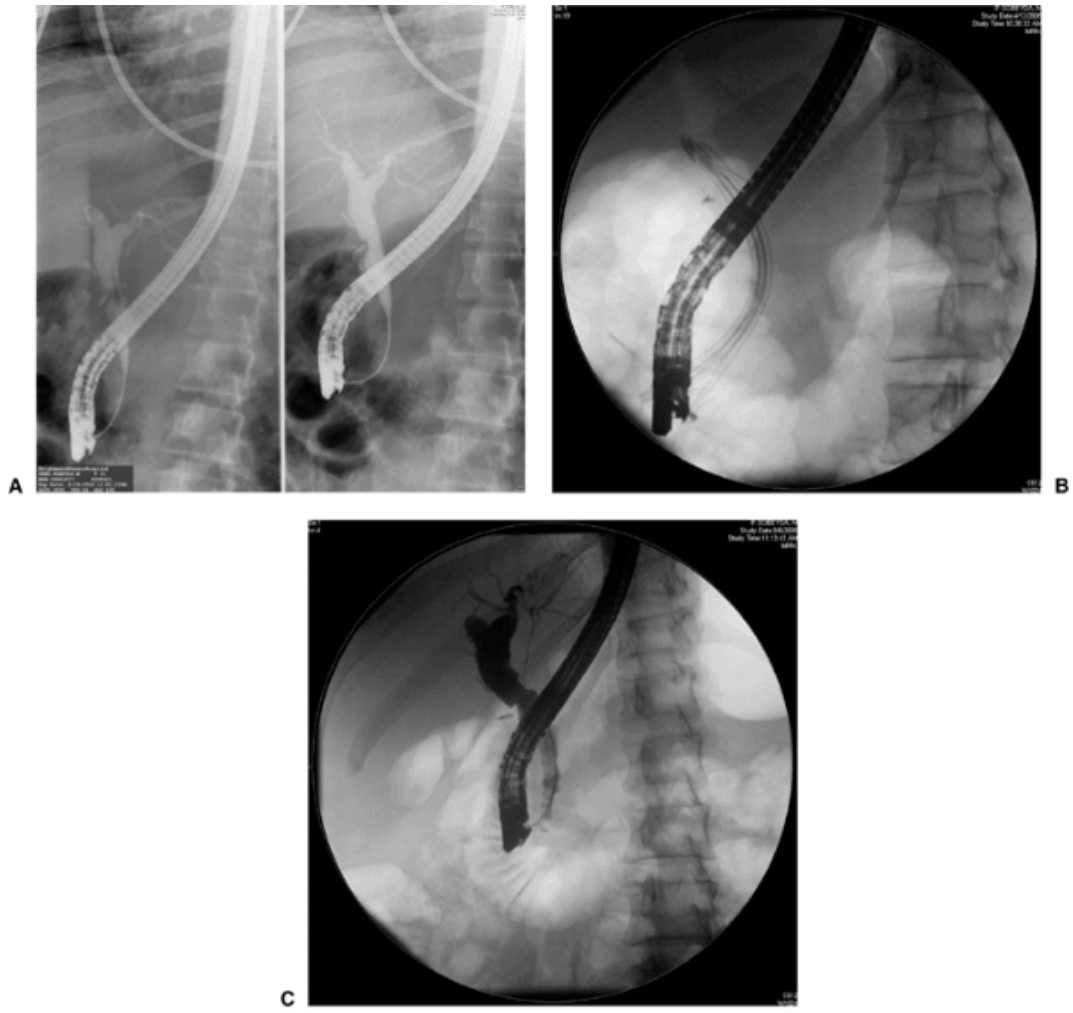
▪ **Figure 6.8 A:** Patient with prior Billroth II partial gastrectomy now with a bile leak after cholecystectomy and a distal bile duct stone; **(B)** short 2-cm 10-Fr diameter plastic stent placed for treating bile leak; **(C)** major common hepatic duct injury showing clips placed partly across the duct and concomitant leak.

Benign bile duct strictures are another complication of laparoscopic cholecystectomy (Fig. 6.9A). Bile duct strictures after laparoscopic cholecystectomy tend to be short (<10 mm) and occur at several well-defined areas: Mid-CBD (42% to 50%), confluence of the right and left hepatic ducts (22% to 41%), common hepatic duct (28%), distal CBD (15%), and cystic duct (20%). Strictures may result from misplacement of a clip or suture during surgery, often across the right hepatic duct or CBD, or after inadvertent ischemic injury to the bile duct, which may evolve over time and, therefore, potentially present late.

Options for therapy include surgery and percutaneous or endoscopic dilatation with stent placement. Surgical management of benign biliary strictures results in a good response in 75% to 93% of patients, with a

stricture recurrence rate of 18% (61). Early surgical referral, no previous repair,

the quality of the proximal duct, and greater distance between the stricture and the confluence are associated with a favorable surgical outcome. Similarly, percutaneous biliary dilatation of benign biliary strictures has a reported success rate between 33% and 100%, with a long-term patency rate of 76% (62). However, several sessions are required to obtain a satisfactory outcome.



▪ **Figure 6.9 A:** Benign bile duct stricture after cholecystectomy in a patient undergoing hydrostatic balloon dilatation; **(B)** four 10-Fr plastic stents placed across the stricture; **(C)** result after 6 months of endoscopic therapy with the resolution of the stricture.

The role of ERCP in the management of benign biliary strictures is well established (63). The guiding principles include clearly defining the stricture, excluding further strictures, and determining the degree of communication between left and right systems. Stricture dilatation is performed with a balloon or with sequential catheter dilators over a guidewire. Previous sphincterotomy eases instrumentation, facilitates placement of multiple stents, and may reduce the incidence of pancreatitis after stenting. After initial dilatation, a single 8- or 10-Fr stent should be placed for an initial period of 6 to 8 weeks to reduce the rate of restenosis (64). Placement of several stents may further reduce the long-term rate of restenosis (Fig. 6.9B). Stent changes every 3 months for up to 1 year are

required to achieve long-term clinical results. Success rates higher than 80% have been reported with this endoscopic regimen (61,62,65). The likelihood of successful endoscopic therapy is based on the age of the stricture. A delay in diagnosis greater than 3 months significantly reduces the success rate (62).

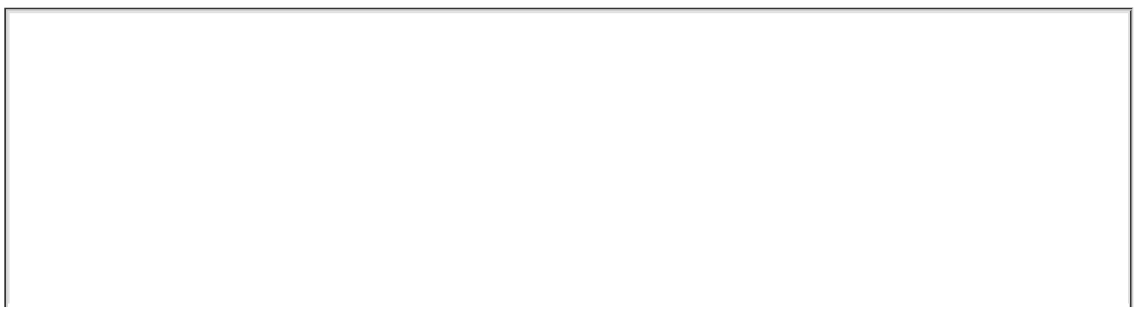
Results of a retrospective study showed similar long-term success rates between endoscopic and surgical interventions (66). These findings suggest that surgery be reserved for patients with complete duct transection, failed previous repairs, failed endoscopic therapy.

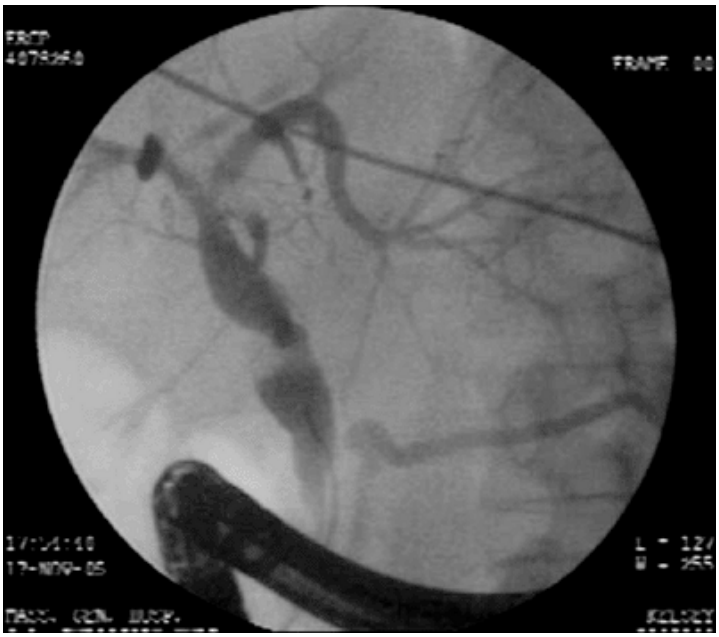
The role of SEMS in the management of benign strictures, particularly postoperative strictures, remains unclear. Although there are reports of successes with SEMS in postoperative biliary strictures, concerns about long-term patency have prevented the standard use of the stents (67,68,69). Furthermore, the relative safety and success of surgery means that in suitable patients it may be a more logical alternative to failed endoscopic management with plastic stents unless SEMS seem the only option, in which case a covered stent would be logical.

Liver Transplantation

ERCP plays several roles in the care of patients in the pre- and posttransplantation settings. Preoperatively, ERCP may be used to evaluate the biliary tree and rule out any concomitant biliary disorder such as choledocholithiasis or cholangiocarcinoma. More commonly, however, ERCP is used after transplantation to diagnose and manage postoperative complications (70,71,72). Biliary tract complications after liver transplantation include bile leaks, which tend to occur early in the postoperative period, and strictures, stones, and sphincter dysfunction, which typically occur later (Fig. 6.10).

The biliary ductal anastomosis most often performed during liver transplantation in adults is an end-to-end choledochocholedochostomy with or without T-tube placement. Roux-en-Y choledochojejunostomy is performed in children or adults if there is evidence of biliary tree disease (e.g., PSC) in the recipient, if there is a considerable size mismatch between recipient and donor (e.g., split graft transplantation), or if the free biliary ends do not lie close enough to each other to allow tension-free anastomosis. Bile leaks can occur at the site of either the ductal anastomosis or T-tube insertion. As with postcholecystectomy leaks, posttranplant bile leaks typically manifest early in the postoperative period as pain, fever, or unexplained elevated results of liver function tests. Several factors can contribute to the development of a bile leak in the postoperative period, including local ischemia, tissue preservation problems, technical difficulties, relative obstruction of distal bile flow by debris, a clogged T-tube, or papillary stenosis (73). The mainstay of management is endoscopic clearance of debris after sphincterotomy, with stent placement if necessary. Surgery is indicated in the setting of massive bile leaks.





▪ **Figure 6.10** Stricture of choledochocholedochostomy after liver transplantation (with acknowledgment to Dr. Peter Kelsey, Massachusetts General Hospital.)

Bile leaks at the anastomosis, at the T-tube removal site, or due to parenchymal injury (e.g., a reduced-size graft in a pediatric patient, a graft from a living-related donor, or a split graft) are successfully treated by placement of a transpapillary stent (64). Unless there is evidence of stone or debris obstruction or of papillary stenosis or suspected SOD, sphincterotomy is not necessary.

Biliary strictures after liver transplantation most commonly occur at the choledochocholedochostomy or choledochoenterostomy anastomosis and may manifest any time in the postoperative period. They may be caused by hepatic arterial thrombosis (a major complication of liver transplantation) or ischemic injury after prolonged organ preservation (cold ischemic time >13 hours). Although strictures typically manifest with the signs and symptoms of biliary obstruction, proximal dilatation of the biliary tree on imaging studies is not always seen, likely due to graft disease. The length and diameter of the anastomotic stricture are predictive of the likelihood of success of endoscopic intervention. Short, mildly narrowed strictures are more likely to resolve after endoscopic dilatation or temporary stenting. Longer or multiple strictures often may not be managed successfully with endoscopy alone and may necessitate either surgical reconstruction or retransplantation for definitive treatment.

Papillary stenosis (SOD) can occur in the post-transplantation period. It typically manifests as cholestasis associated with a tightly narrowed terminal CBD segment and proximal dilatation. ES is the treatment of choice.

Choledocholithiasis occurs sporadically in the posttransplantation period. Biliary cast syndrome is a rare form of choledocholithiasis in which sludge and sloughed necrotic biliary epithelial cells or clot form casts within the bile duct. Preservation or ischemic

injury is thought to be the main etiology. Biliary casts may be extracted by ERCP, but an open procedure may be necessary for extensive disease.

Pretransplantation liver disease, particularly PSC, may recur after liver transplantation. Disease recurrence is often difficult to differentiate from ischemic strictures or opportunistic infections in the biliary tract. Recurrent PSC may be suspected when a pattern of multiple strictures in a well-preserved graft is encountered with no apparent vascular or infectious problems. In patients with PSC, the possibility of a malignant stricture should be considered, especially if there is unsuspected cholangiocarcinoma in the explant. Unfortunately, because these patients often undergo Roux-en-Y hepaticojejunostomy as part of the transplantation procedure, an endoscopic approach may be impractical.

Other Postoperative Injuries

Biliary strictures and leaks can also develop after other hepatic, biliary, or pancreatic resections with reimplantation of the bile ducts. Biliary leaks can be successfully managed with stent therapy (74). Although amenable to both dilatation and stenting, strictures may be difficult to access with endoscopy because of surgically altered anatomy or distortion by liver atrophy or hypertrophy (75).

Other Benign Biliary Strictures and Injuries

Trauma

A variety of nonsurgical causes of bile duct injury may necessitate endoscopic management. There have been several reports of successful endoscopic therapy for biliary fistulas resulting from both blunt and penetrating liver trauma (76). Success, however, appears to depend on the nature and location of the injury and the patient's clinical status. Endoscopic therapy, including stent placement, has been described in the successful closure of a fistula between a transjugular intrahepatic portosystemic shunt and the biliary ducts, and in the management of bile leak secondary to liver biopsy and penetrating trauma (77).

Iatrogenic Strictures

Ischemic bile duct strictures have been associated with hepatic arterial infusion of 5-fluorodeoxyuridine (78). Therapy includes withdrawal of the drug and surgical, percutaneous, or endoscopic drainage of the dilated biliary tree. Direct toxic injury to the biliary tree after using formaldehyde or alcohol solutions in the treatment of hydatid disease has also been described.

Chronic Pancreatitis

Biliary strictures accompany chronic pancreatitis in as many as 29% of cases (79). These strictures tend to be relatively long (mean length, 3.9 cm). Clinical manifestations include abdominal pain (65%), jaundice (43%), secondary biliary cirrhosis (15%), and cholangitis (15%). Surgical bypass with choledochoduodenostomy or choledochojejunostomy has been the procedure of choice but is associated with a high morbidity (25%) in alcoholic patients. Endoscopic stent placement is a safe and potentially beneficial alternative therapy. Single biliary stents have shown short-term success in maintaining biliary drainage (80,81) and may even induce the reversal of secondary hepatic

fibrosis (82). Multiple stents may prove more successful than single stents (83). SEMS have also shown preliminary success in maintaining biliary drainage in chronic pancreatitis (84,85,86), but long-term studies of efficacy and safety have not yet been published.

Malignant Biliary Obstruction

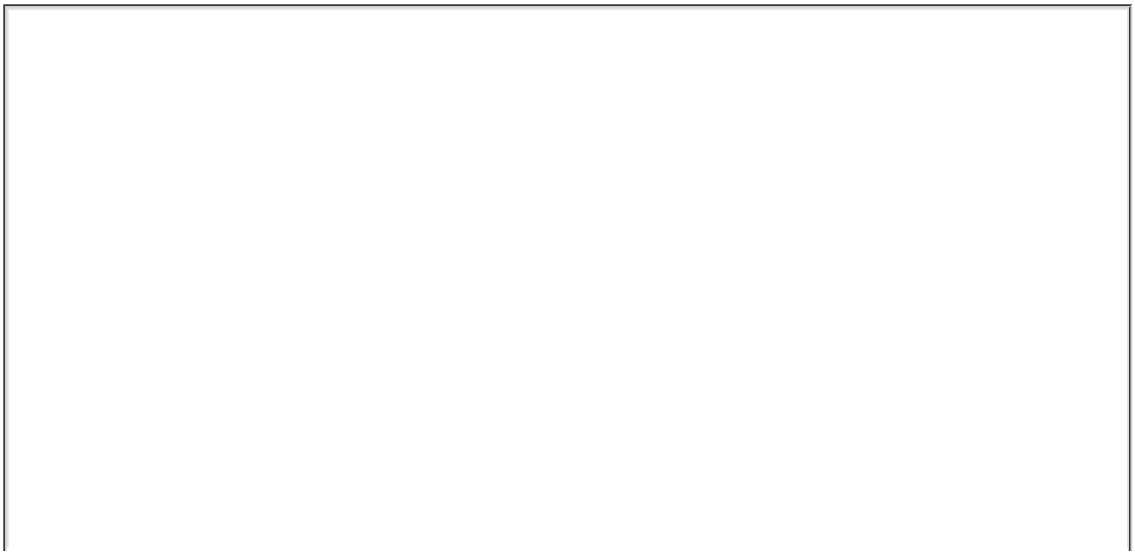
Diagnosis and Staging

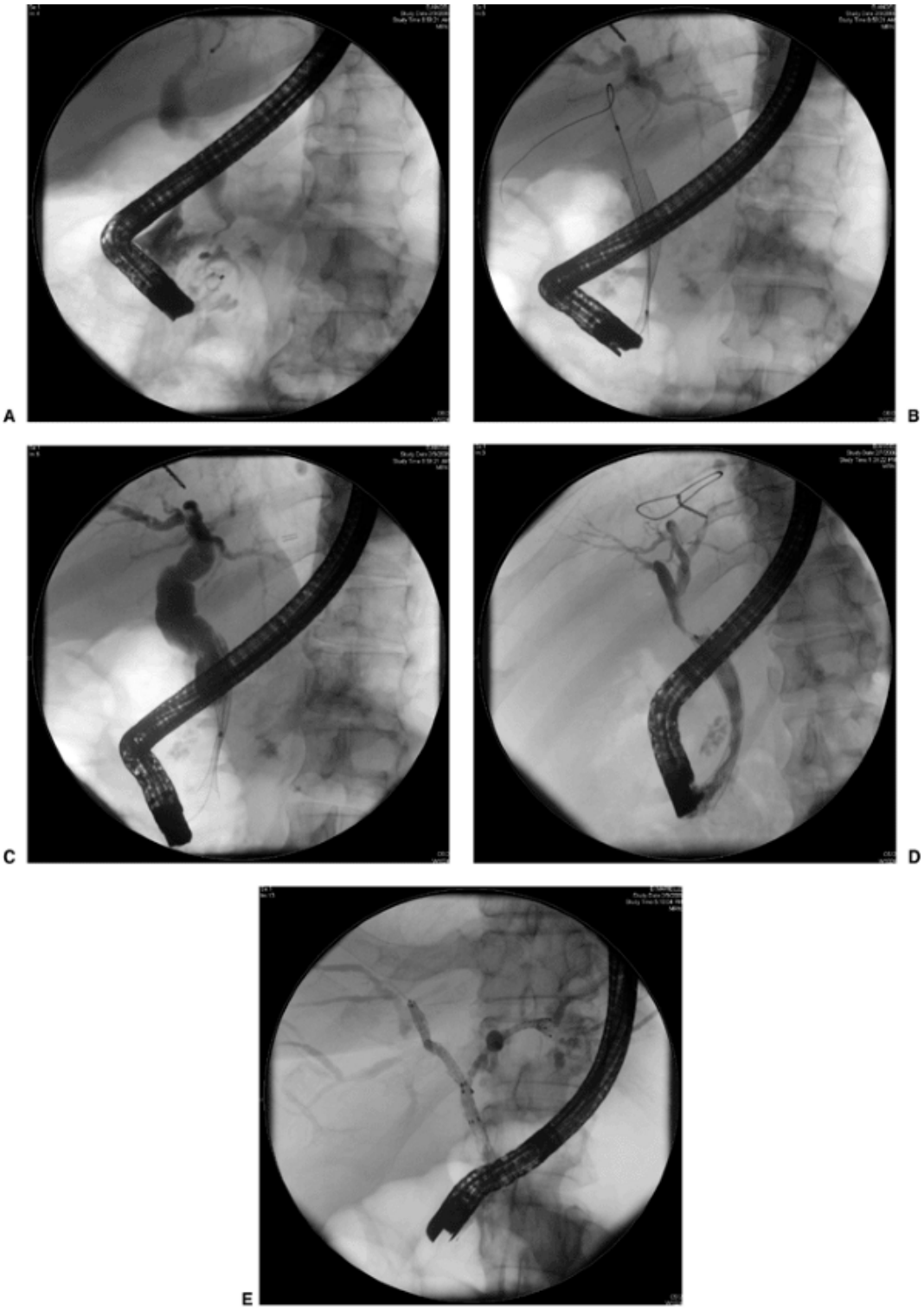
Malignant biliary obstruction is caused by tumors arising in the biliary tree (including papilla) or adjacent organs (e.g., pancreas) or by metastatic lesions. Patients with malignant biliary obstruction usually have progressive cholestasis and pruritus, but fever is rare in the absence of previous intervention.

Signs of biliary dilatation are evident on imaging by ultrasound, MDCT, or MRCP, but the malignancy itself may not be identified easily. Patients with tumors arising in the major papilla or pancreas usually have dilatation of both pancreatic and biliary ducts. The “double-duct” sign (stricture and dilatation of both the biliary and pancreatic ducts) is highly suggestive of a lesion of the head of the pancreas.

ERCP has many roles in differentiating malignant from benign causes of biliary obstruction. Conventional biopsy of lesions involving the papilla or duodenum can be performed in the appropriate setting. After identifying the stricture on cholangiography, a cytology brush can be inserted into the strictured area to obtain specimens for analysis. The sensitivity of brush cytologic examination is highest for primary bile duct tumors, intermediate for pancreatic cancers, and lowest when biliary obstruction is secondary to metastatic malignant disease (87). The sensitivity of brush cytology increases from 40% for one brushing to 62% for three brushings in documented cases of cholangiocarcinoma (88). A small biopsy forceps can be passed under direct visualization (with cholangioscopy) or fluoroscopy into ductal strictures. The overall sensitivity

of cytology increases to 80% when biopsy is used in combination with cytologic brushing (62). Cytologic examination of aspirated bile has the lowest diagnostic sensitivity—34% to 50%. Newer devices for tissue acquisition, including tissue scrapers, are being evaluated and may improve the sensitivity for malignant disease. Bile tumor markers, including K-ras and p53, are under investigation.





▪ **Figure 6.11 A:** Malignant distal bile duct stricture; **(B)** appearance immediately after deployment of a self-expanding metallic stent; **(C)** injection of contrast through the stent to document patency and appropriate position; **(D)** partial occlusion of metallic stent by tumor ingrowth; and **(E)** three metallic stents used to palliate hilar malignancy affecting the right and left hepatic ducts.

Although ERCP is not designed for staging malignant disease of the pancreatic or bile duct, the development of intraductal ultrasonography, in which a high-frequency ultrasound probe is introduced into the bile duct or pancreatic duct through a duodenoscope, may prove to be an important tool in staging early and, possibly, resectable cases of malignant biliary obstruction (89).

Endoscopic Therapy

With malignant obstruction, the major role of ERCP is in the achievement of biliary drainage through plastic or metal stents. Duodenoscopes with larger channels (3.7 and 4.2 mm) are used to deploy 10- and 11.5-Fr plastic stents and SEMS (Fig 6.11A). Technical success rates of 90% have been reported. Inability to gain access to the papilla because of tumor occlusion of the duodenum, surgically altered anatomy (Billroth II or Roux-en-Y anastomosis), or the inability to pass a guidewire across the stricture are the most common causes of technical failure. Endoscopic management of malignant biliary obstruction is superior to both surgical bypass and percutaneous stent placement (90,91). However, in cases in which an attempt at surgical resection is anticipated, preoperative biliary drainage by ERCP has no proven advantage except when there has been previous instrumentation (92,93,94).

Dilatation of distal malignant biliary strictures is usually not necessary for stent deployment, but may be necessary for high-grade or hilar strictures (Fig. 6.11E). If endoscopic attempts are unsuccessful, percutaneous transhepatic cholangiography is used to access the stricture and to place a percutaneous biliary drain through it over a guidewire. The biliary drain can then be replaced with a stent through either percutaneous or endoscopical means. Some experts recommend prophylactic administration of antibiotics to all patients with obstruction, whereas others have argued for selective use of antibiotics when drainage is not established (95).

At the time of the initial ERCP, plastic biliary stents are inserted to allow for diagnosis and tumor staging if these are not already available. Biliary stents are not permanent and require replacement. The optimal time to replace these stents is generally between 3 and 6 months based on low occlusion rates with 10- and 11.5-F stents at 3- and 6-month intervals, respectively (96). An alternative approach is based on close clinical follow-up evaluation and exchange of the stent when there is evidence of occlusion (i.e., low-grade fever, malaise, or dark urine). It is our practice to change biliary stents at 3- to 4-month intervals.

If a malignancy is deemed unresectable, SEMS are used for palliation. When compared to plastic stents, metal stents have better stent patency and are more cost-effective (97,98). Several expandable metal stents are available for use in the biliary tract. These stents can be delivered from a small-caliber (e.g., 8-Fr) catheter system and once released can expand up to 10 mm in diameter. For malignant obstruction of the distal bile duct, the tip of the stent is usually left outside the papilla. For more proximal biliary lesions (e.g., within the common hepatic duct), it is possible for the deployed stent to be laid entirely within the bile duct. For malignant obstruction, the technical success rates for biliary placement of SEMS have been reported to be as high as 95%, with few immediate complications (69). Metal stents can become obstructed by tumor ingrowth or overgrowth and more rarely by sludge and bacterial adherence. These stent blockages can be relieved by diathermic devices, extraction balloon catheters, or the insertion of a standard plastic stent or a second metal stent. Coated SEMS have been developed and may confer the advantage of prolonged patency

compared to uncovered stents.

Adjuvant Therapy

The delivery of adjuvant therapy for malignant biliary obstruction has been investigated. Intrabiliary irradiation has been performed on an investigational basis with iridium 192 wires placed by the percutaneous or endoscopic route. More recent work has focused on the delivery of photodynamic therapy to the bile duct through an ERCP-guided catheter. The use of hematoporphyrin derivatives as sensitizers, followed by intraluminal photoactivation, showed mixed results in producing prolonged biliary decompression, improved survival, and improved quality of life for patients with advanced bile duct cancer (99,100,101).

Other Biliary Disease Infections

Acquired Immunodeficiency Syndrome Cholangiopathy

Patients with human immunodeficiency virus (HIV) infection appear to be uniquely susceptible to disease in the biliary system, because profoundly immunosuppressed transplant recipients are not similarly predisposed. The syndrome that has been termed *AIDS cholangiopathy* clinically describes the condition of

patients with HIV infection, who usually have CD4 counts less than 200/µL, and right upper quadrant pain, dramatic elevation in alkaline phosphatase level (mean value 800 IU/L), and modest hyperbilirubinemia (mean value 1.5 mg/dL) in the absence of stone disease or tumor obstruction (43). Numerous opportunistic pathogens have been found in the bile ducts of these patients, including *Cryptosporidium*, cytomegalovirus, and *Microsporidia* such as *Enterocytozoon bieneusi.* On ERCP, the appearance of the bile duct can vary from classic papillary stenosis with a dilated CBD that tapers distally and markedly retains contrast material, to intrahepatic or extrahepatic sclerosing cholangitis. The response to ES is similarly variable. Patients whose cholangiograms reveal papillary stenosis appear to have a dramatic reduction in pain after sphincterotomy, although levels of alkaline phosphatase remain elevated (102). The role of endoscopic therapy in cases of more diffuse involvement has not been established.

Parasitic Infection

Echinococcosis, caused by the small tapeworms *Echinococcus granulosus* and *Echinococcus multilocularis* is typically managed surgically, but when it is associated with biliary obstruction, ERCP can help in the management. Large hydatid cysts located near the porta hepatis in the liver can cause obstruction by external compression. Smaller hepatic cysts also can rupture into bile ducts and form biliary fistulas. The hydatid membranes can then obstruct the bile duct. These biliary obstructions can be defined by ERCP, and sphincterotomy can lead to successful extraction of hydatid material from the CBD (103,104) or to the resolution of the fistulas.

The biliary manifestations of ascariasis, caused by the large roundworm *Ascaris lumbricoides*, are a result of the worm's propensity to enter narrow orifices such as the papilla. Previous ES is a predisposing factor (105). Migration of the worm into the bile ducts can result in biliary obstruction and acute or chronic

cholangitis. Intraductal worm death may result in fibrosis, stricture, and stone formation. Combined with medical antihelminthic therapy, endoscopic extraction of the worm with a forceps, basket, or snare can be therapeutic.

Fasciola hepatica, a large, hermaphroditic trematode, invades the biliary tract during the acute invasive phase, when the parasite migrates through the liver, and the chronic phase, when the parasite matures and resides in the biliary tract. During the chronic phase, biliary colic, jaundice, and even hemobilia can occur. Worms as large as 13 to 30 mm can be visualized on ERCP. Although fascioliasis can be managed medically, sphincterotomy and balloon extraction of the parasites have also been effective (106).

Liver flukes migrate through the papilla and into biliary ducts, pancreatic ducts, and the gallbladder before ultimately residing in the smallest biliary duct that can accommodate them. *Clonorchis sinensis* is the most common fluke in humans, followed by *Opisthorchis* species. The infection is often asymptomatic but can result in cholestasis, secondary biliary cirrhosis, or superimposed bacterial cholangitis. The presence of liver flukes causes marked proliferation of the biliary epithelium and is associated with an elevated risk of cholangiocarcinoma. ERCP is useful in the diagnosis because it shows filling defects and tapering of the terminal bile ducts, often with a characteristic pattern of multiple cystic dilatations. These cystic dilatations may persist on cholangiograms despite successful medical therapy with praziquantel.

Pediatric Endoscopic Retrograde Cholangiopancreatography

Success and Complications

ERCP in neonates and infants is now possible using specialized smaller duodenoscopes. ERCP in neonates is technically demanding, but the procedure in older children is no more difficult than it is in adults (107). Cannulation rates in neonates and infants are lower than those in adults, ranging from 27% to 95% (107), but comparable in children older than 1 year. The overall complication rate (mainly from pancreatitis) in the pediatric population ranges from 5% to 7%. The same medications are used for conscious sedation but the doses are age adjusted. Some centers use general anesthesia routinely. Lead protection of nonbiliary areas, judicious limitation of fluoroscopy, and efficient endoscopic practices are necessary to minimize radiation exposure.

Indications for Endoscopic Retrograde Cholangiopancreatography in Neonates and Children

The biliary indications for ERCP in neonates and children are listed in Table 6.5. The most important role of ERCP in neonates is in the evaluation of neonatal cholestasis, in which up to 80% of cases are either extrahepatic biliary atresia or neonatal hepatitis. An early diagnosis is crucial because extrahepatic biliary atresia requires early corrective surgery (Kasai operation or hepatoportoenterostomy) to achieve a favorable outcome. In the initial evaluation of neonatal jaundice, ERCP has a sensitivity of 83% to 86% for the diagnosis of biliary atresia and of 93% to 100% for the diagnosis of neonatal hepatitis (108). Biliary atresia has been classified into three categories (78). In type 1, the

biliary tree is not visualized. In type 2, the distal CBD and the gallbladder can be

seen, but the main hepatic and intrahepatic ducts cannot. Type 3 is divided into two subtypes: Type 3a, in which the gallbladder and the complete common duct are visualized with biliary lakes at the porta hepatis, and type 3b, in which both hepatic ductal systems have lakes.

Table 6.5. Biliary Indications for Endoscopic Retrograde Pancreatography in a Pediatric Population

Congenital anomalies
Biliary atresia vs. neonatal hepatitis
Paucity syndrome
Congenital hepatic fibrosis
Biliary strictures due to cystic fibrosis
Congenital biliary strictures
Choledochal cyst
Acquired diseases
Primary sclerosing cholangitis
Biliary obstruction due to parasitic infestation
Cholelithiasis and choledocholithiasis
Biliary obstruction or leaks after liver transplantation
Malignant obstruction of common duct

Another variant of biliary atresia is characterized by a paucity or absence of intrahepatic biliary ducts with a normal or hypoplastic extrahepatic duct. This condition may occur as part of Alagille syndrome. It is important to differentiate this condition from extrahepatic biliary atresia to avoid unnecessary surgery. Another condition to consider with a normal CBD and irregular intrahepatic ducts is congenital hepatic fibrosis. This is a recessive genetic disorder characterized by multiple intrahepatic macroscopic and microscopic bile duct cysts within bands of fibrous tissue.

As in adults, PSC in children is associated with inflammatory bowel disease and other autoimmune disorders. PSC in children can be accurately diagnosed by pruning the peripheral biliary tree and irregular areas of stenosis and ectasia during cholangiography. Endoscopic treatment with balloon dilatation of strictures and clearance of any obstruction results in clinical and laboratory improvement.

Cystic fibrosis is characterized by dilatation, narrowing, and beading of the intrahepatic ducts secondary to focal or multilobular biliary cirrhosis. These abnormalities have been attributed to intrahepatic mucus plugging.

Cholelithiasis is rare in childhood, except for certain ethnic groups, but is associated with chronic hemolytic anemia, short bowel syndrome, congenital anomalies of the biliary tree, cystic fibrosis, neonatal sepsis, prolonged parenteral nutrition, or chronic bile acid malabsorption. Choledocholithiasis occurs only rarely in infants and children and may be associated with biliary tract malformations such as choledochal cysts, chronic liver disease, hemolysis, and infection. Ultrasound and MRCP are often the initial diagnostic procedure. ERCP serves a confirmatory and therapeutic role. ES is the procedure of choice in the management of retained and recurrent bile duct stones and has been performed safely (109).

Choledochal cysts are a congenital malformation of the biliary tract that usually manifest before the patient is 10 years old (110). Patients may present with jaundice, cholangitis, abdominal pain, a palpable mass, or failure to thrive. The classic triad of jaundice, abdominal pain, and a palpable mass is present in only a minority of patients. Type I, the most common, is characterized by the dilatation of the CBD. Type II is a true diverticulum of the CBD. Type III is known as a *choledochocele* and occurs at the papilla. Type IV is characterized by multiple cysts. In type IVA, the cysts are both intra- and extra-hepatic. Type IVB has only extrahepatic cysts. Type V is also known as *Caroli's disease* and is characterized by intrahepatic cysts. ERCP is used to define the biliary anatomy, including any anomalous union between the CBD and the pancreatic duct that may contribute to the pathogenesis of the cysts. ERCP is also used to treat complications of the cysts including CBD stones and sludge. Given a higher risk of biliary malignancies in patients with choledochal cysts, the treatment of these cysts is typically surgical resection, except for cholodochoceles, which do not confer an increased risk of malignancy and can be treated by ES.

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Chapter 7
Physioanatomic Considerations

Ian R. Wanless

Key Concepts

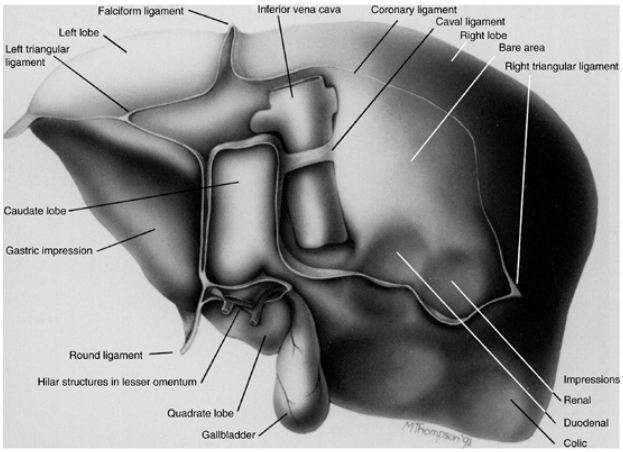
- The branching pattern of large vessels and ducts is used for defining the segmental nomenclature used by radiologists and surgeons.
- Anomalies of the vessels and ducts are of importance to surgeons.
- Bile ducts are supplied by arteries. Arterial injury may lead to ischemic strictures of the ducts, especially after transplantation.
- The liver receives most of the splanchnic blood flow. After severe obstruction at any level of the hepatic vasculature, collateral channels become a source of bleeding and are partly responsible for hepatic encephalopathy.
- The microvasculature of the liver consists of small branches of portal and hepatic veins that interdigitate in a regular pattern, allowing the definition of parenchymal subunits called *acini* or *lobules*.
- Arterioles communicate with portal veins through the periportal end of the sinusoids. In cirrhosis, these communications dilate and, therefore, contribute to portal hypertension.
- Hepatocytes are exposed to gradients of nutrients and waste products, leading to zonal metabolic specialization and zonal variation in the susceptibility to ischemia and drug toxicity.
- The sinusoids are lined by fenestrated endothelial cells that lack basement membranes. This anatomy facilitates rapid exchange between plasma and hepatocytes.
- Hepatic lymph is formed at the sinusoidal level when there is increased sinusoidal pressure, especially with outflow obstruction. This lymph may accumulate as ascites.
- Sinusoidal macrophages (Kupffer cells) are important in host defense.
- Perisinusoidal stellate cells store vitamin A and, when activated, produce collagen that contributes to the pathogenesis of cirrhosis.
- There are two major anatomic forms of chronic liver disease: Cirrhosis and nodular regenerative hyperplasia.
- Nodular regenerative hyperplasia occurs when multiple acini undergo ischemic atrophy and the less-affected acini undergo compensatory hyperplasia.
- Cirrhosis develops when the stromal habitat of hepatocytes is damaged and replaced by dense collagen. The topographic distribution of the resulting parenchymal extinction correlates exactly with the obliteration of small veins.

Anatomic knowledge is required for understanding the normal hepatic physiology and the pathogenesis of disease. This chapter presents a summary of normal anatomy, some physiologic correlates, and a description of the major anatomic abnormalities found in human liver disease. This chapter represents an evolution of the legacy provided by Dr. Aron Rappaport in the earlier editions of this book. Material of current general interest has been retained in this edition. Earlier editions may be consulted for citations of historical interest.

Surface Anatomy

The liver is shaped like a wedge, with its base against the right abdominal wall and its tip pointing to the spleen. The normal liver extends from the fifth intercostal space in the midclavicular line down to the right costal margin. It measures 12 to 15 cm coronally and 15 to 20 cm transversely. The lower margin can usually be felt below the costal margin during inspiration.

Transcutaneous puncture for liver biopsy is commonly located in the midaxillary line in the third interspace below the upper limit of liver dullness during full expiration, commonly in the ninth intercostal space. The median liver weight is 1,800 g in men and 1,400 g in women (1). The adult liver weight is between 1.8% and 3.1% of body weight in 80% of individuals (2,3). Liver weights in fetuses and children are relatively greater, being 5.6% at 5 months gestational age, 4% to 5% at birth, and 3% at 1 year of age (4,5).



• **Figure 7.1** Posterior view of the liver. The marks impressed on the liver surface by neighboring organs mirror its topographic relations. (Drawn by M. Thompson.) (From Wanless IR. Anatomy and developmental anomalies of the liver. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger and Fordtran's gastrointestinal and liver disease*, 6th ed. Philadelphia: WB

Saunders, 1997:1056, with permission.)

Impressed by its molding against adjacent organs, William Osler quipped that the liver was present only for packing purposes. Therefore, the superior, anterior, and lateral surfaces are smooth and convex to fit against the dome of the diaphragm. The muscle bundles of the diaphragm often impress grooves in the superior surface. The costal margin often marks a transverse groove on the anterior surface (corset deformity). The posterior surface has indentations from the colon, kidney, and duodenum on the right and from the stomach on the left (Fig. 7.1). Deeper grooves, called *fissures*, are formed where extrahepatic vessels or cords press against the developing liver. Three of these structures, the umbilical portion of the left portal vein (PV), the ductus venosus (ligamentum venosum), and the umbilical vein (ligamentum teres), form the umbilical fissure.

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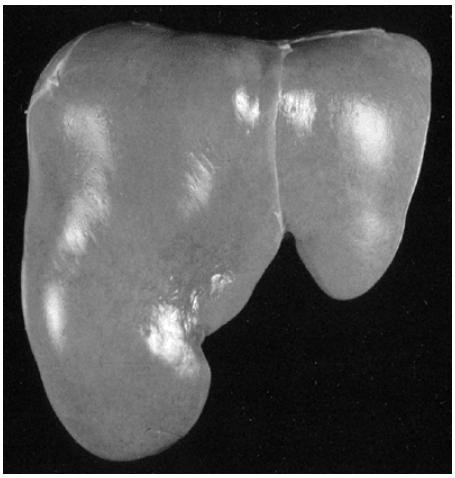
The liver is covered by the fibrous capsule of Glisson (or Walaeus). At the porta hepatis, the connective tissue of the capsule is continuous with the fibrous sheath, which invests the portal vessels and ducts and follows them to their smallest ramifications. The capsular peritoneum reflects onto the diaphragm and continues as the parietal peritoneum. The reflections form the coronary ligaments, the right and left triangular ligaments, and the falciform ligament (Fig. 7.1). These ligaments hold the liver firmly in its place and allow the passage of lymphatics, small vessels, and nerves. There is a large bare area where the liver is attached to the diaphragm and retroperitoneum. The vena cava, being retroperitoneal, lies on the bare area and is held to the liver by a ligament or bridge of the liver parenchyma between the caudate and right lobes.

The falciform ligament connects the liver to the diaphragm and anterior abdominal wall. The lower free edge of the falciform ligament, called *the round ligament*, contains the obliterated umbilical vein. The falciform ligament ascends the anterior surface of the liver, joins the reflections of the peritoneum left of the vena cava, continues posteriorly as the lesser omentum in the fissure of the ductus venosus, and finishes at the hilum. Therefore, the falciform ligament, anteriorly, and the lesser omentum and umbilical fissure, posteriorly, divide the liver into the conventional right and left lobes.

On the posterior surface, the transverse portal fissure contains the hilar vessels and demarcates the conventional right lobe anteriorly from the caudate lobe posteriorly (Fig. 7.1). The quadrate lobe is the portion of the right lobe anterior to the transverse fissure and is delimited on the right by the gallbladder and on the left by the umbilical fissure.

The hepatoduodenal ligament connects the liver to the superior part of the duodenum. It is part of the lesser omentum, which sheathes the hepatic artery (HA), PV, nerves, bile duct, and lymph vessels, all being present within the porta hepatis. In the ligament the common bile duct lies to the right, the HA to the left, and the PV behind them. However, variations in the topography of the HA are common.

There are several variations in the gross anatomy and topography of the liver (6,7). The relative size of the right and left conventional lobes is variable, being equal in size in 7% of individuals and greater on the left in 4% (7). Riedel's lobe is a caudal prolongation of the right lobe, which may give a false impression of hepatomegaly (Fig. 7.2). The falciform left lobe is an elongated lobe that extends laterally and posteriorly like a scythe, found in 19% of individuals (7). Extreme atrophy of the left lobe (4%) may be a result of vascular anomalies occurring early in life (8) or the extinction of parenchyma occurring after acquired vascular obstruction. The left lobe may be attached to the rest of the liver by a narrow pedicle. Accessory livers may be found in the ligaments or mesentery or on the surface of the gallbladder, spleen, or adrenals (6).



• **Figure 7.2** Liver with Riedel's lobe, a prominent caudal extension of the right lobe.

Segmental Anatomy

Division of the liver at the falciform ligament and umbilical fissure does not correspond to the division based on branch points in the vascular supply. Surgical imperative has led to the search for functional divisions within the liver. The anatomic studies of Rex and others (9,10,11,12) demonstrated that the liver can be divided on a different plane into right and left livers (or hemilivers), each with its own blood supply and duct drainage. The right hemiliver comprises 50% to 70% of the liver mass. The liver can be further divided into a total of eight segments on the basis of the vascular or bile duct distribution (7,12,13,14,15,16) (Figs. 7.3 and 7.4). The segmental nomenclature devised by Couinaud has received the widest acceptance. This classification was based on the divisions of the PVs. However, the branching of the PVs to the left lobe is irregular because of the entry of the umbilical vein, making it desirable to adopt a nomenclature based on the divisions of the arteries or ducts, as suggested by Strasberg (17). This can be done without modifying the segments defined by Couinaud and rationalizes the diverse nomenclature used in different parts of the world. The Strasberg nomenclature is summarized in Figures 7.3 and 7.4 and Table 7.1.

Most hepatic resections can be achieved by division either on Cantlie's line (between the gallbladder

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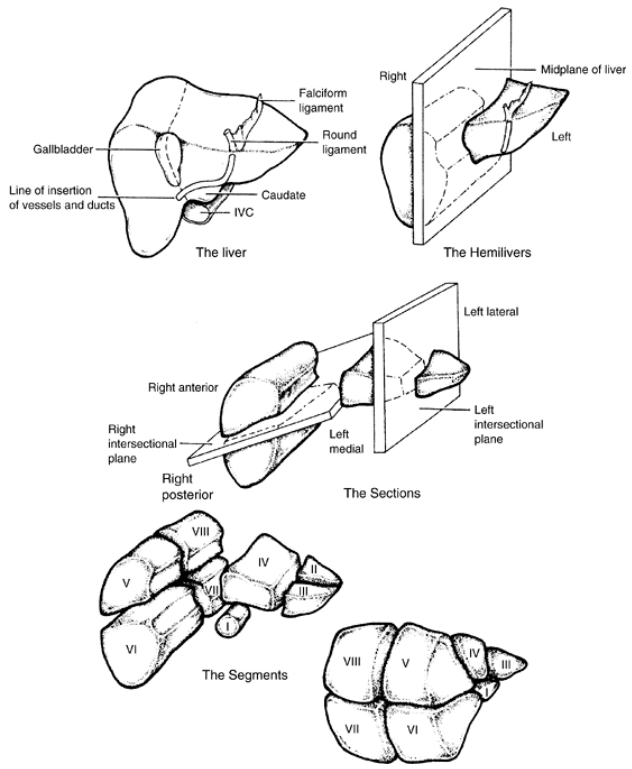
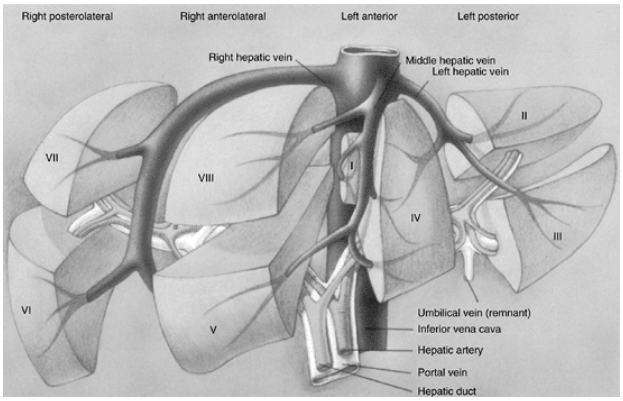
and vena cava) or near the falciform ligament. Surgical dissection along the planes between segments is relatively bloodless. Because the segments do not have surface landmarks, small resections are usually performed without attempting to identify the segmental

boundaries (16). The segments vary greatly in size and shape among individuals (18), so that each operation is empirical and may be based on ultrasonography (19,20)



• **Figure 7.3** Schematic diagram of the planes of division in the liver. The liver can be visualized as being divided into two hemilivers by the midplane of the liver. The hemilivers are each subdivided into two sections by right and left intersectional planes. Three of the sections are further subdivided into two segments each by intersegmental planes on the basis of the divisions of the ducts and arteries. The left medial section does not have a regular duct and artery division and is therefore called *one segment* (IV). However, for surgical convenience, it is subdivided into posterior and anterior portions (segments IVa and IVb, respectively, not shown). The caudate lobe is a separate segment (I) that is not part of the four main sections. IVC, inferior vena cava. (Courtesy of Dr. Strasberg.) (From Strasberg SM. Terminology of liver anatomy and liver resections: coming to grips with hepatic Babel. *J Am Coll Surg* 1997;184:413–434, with permission.)



• **Figure 7.4** Schematic demonstration of the vascular relations of the segments (drawn by M. Thompson). The segments are numbered using the nomenclature of Couinaud. The remaining elements of nomenclature are those of Strasberg. The midplane extends along Cantlie's line from the vena cava to the gallbladder. The middle hepatic vein runs in this plane. The right and left intersectional planes contain the right and left hepatic veins, respectively. Each section is supplied by one of the four major arteries and bile ducts. The portal pedicles and hepatic veins interdigitate, so they do not lie in the same planes except for the umbilical portion of the left portal vein and the umbilical vein (a medial branch of the left hepatic vein), both of which are found in the umbilical fissure (also known as left intersectional plane). The *sections* of Strasberg coincide exactly with the *segments* of Healey and Schroy. The two *sections* of the right hemiliver correspond to the two right *sectors* of Couinaud. The tertiary structures of Strasberg and of Couinaud are called *segments*; these coincide with the *areas* of Healey and Schroy, except that segment IV is divided into two *areas* by these authors. (Modified from Wanless IR. Anatomy and developmental anomalies of the liver. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger and Fordtran's gastrointestinal and liver disease*, 6th ed. Philadelphia: WB Saunders, 1997:1056, with permission.)

Embryology

The liver arises from the hepatic diverticulum of the foregut during the fourth week of gestation (7,21) (Fig. 7.5). As the embryo develops, the blood supply to this region evolves in an elaborate manner to deliver nutrients from three different sources in sequence: Yolk sac, placenta, and gut (7,17).

Hepatocyte precursors, the hepatoblasts, arise from endodermal cells at the advancing front of the diverticulum and invade the mesoderm of the caudal portion of the septum transversum. The vitelline veins traverse the region, bringing blood from the yolk sac and the digestive tube to the heart. As hepatoblasts invade the mesenchyme, they disrupt the vitelline veins, tapping their blood supply. This supply is from the vitelline veins, segments of which later become the PV The hepatic bud is subdivided into cords by new capillaries called *sinusoids*. The sinusoidal flow coalesces into three major hepatic veins. At the time the main hepatic veins are developing, the entire liver is composed of only two lobules, and there is no artery and no left or right bile duct. As the hepatic veins and PVs begin to branch, the branches interdigitate to remain equidistant from each other, and the parenchyma is subdivided into numerous lobules or acini. It has been suggested that the portal and hepatic vessels invade the most ischemic parenchyma that is located at the nodal point of Mall, the point most distant from both PVs and hepatic veins (22,23).

The hepatoblast cords develop into anastomosing tubular structures with central bile canaliculi that

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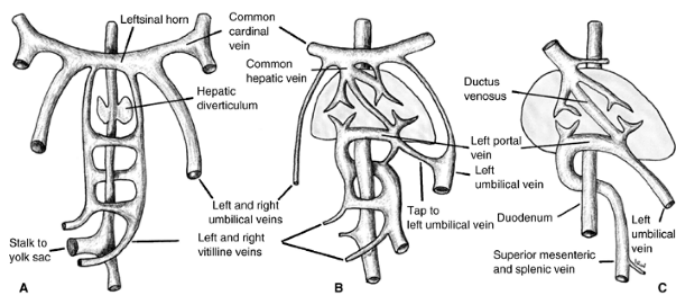
eventually communicate with the bile ducts. Most hepatoblasts differentiate into hepatocytes, but those adjacent to the portal mesenchyme differentiate into a layer of duct progenitors called the *ductal plate* (24,25). The ductal plate becomes bilayered and gradually forms segments with lumina. These segments form ducts that migrate away from the limiting plate to a more central location in the portal tracts near the PV. Portions of the ductal plate resorb, leaving a complex anastomosing network of ducts that continues to simplify in the weeks after birth (26). The common bile duct, left and right hepatic ducts, and gallbladder develop in the stalk region of the hepatic diverticulum. These ducts are continuous with the ductal plate in the cranial end of the diverticulum. As bile ducts develop, they become highly vascularized by arterioles and capillaries of the periductal plexus.

Table 7.1. Nomenclature for Resections of Liver

	Name of operation	
Portion of liver excised	**Strasberg**	**Couinaud, Goldsmith, and Woodburne**
Single segment	Segmentectomy (e.g., segmentectomy III)	—
Two adjacent segments	Bisegmentectomy (e.g., bisegmentectomy V, VIII)	—
Multiple segments	Segmentectomy (e.g., segmentectomy IV, V, VI)	—
One fourth of liver (e.g., left lateral section)	Left lateral sectionectomy	Left lobectomy (segments II and III), left lateral segmentectomy
One half of liver, right hemiliver	Right hemihepatectomy (may or may not include segment I [e.g., right hemihepatectomy with segment I[a]])	Right hepatectomy (segments V, VI, VII, and VIII), right hepatic lobectomy
One half of liver, left hemiliver	Left hemihepatectomy	Left hepatectomy (segments II, III, and IV), left hepatic lobectomy
Three fourths of liver, right hemiliver, and left medial section	Right trisectionectomy or right hemihepatectomy with left medial sectionectomy	Right lobectomy (segments IV, V, VI, VII, VIII, ± I), extended right hepatic lobectomy, right trisegmentectomy (Starzl)
Three fourths of liver, left hemiliver, and right anterior section	Left trisectionectomy or left hemihepatectomy with right anterior sectionectomy	Extended left hepatectomy, extended left lobectomy, left trisegmentectomy (Starzl)

[a]This comment also applies to left hemihepatectomy and the trisectionectomies.
Modified from Strasberg SM. Terminology of liver anatomy and liver resections: coming to grips with hepatic babel. *J Am Coll Surg* 1997;184:413–434, with permission.



• **Figure 7.5** Drawing to show three stages in the development of the hepatic vasculature. **A:** In the embryo, there are three paired venous beds that drain the placenta (umbilical veins), yolk sac, and intestinal tract (omphalomesenteric or vitelline veins), and the remainder of the body (cardinal veins). These beds converge on the sinal horns before entering the heart. The left and right vitelline veins are joined by three anastomoses to form a ladder-like structure with the intestinal tract intertwined. The extrahepatic portal vein develops from these vessels after selective obliteration of portions of the ladder **(B and C)**. **B:** The left vitelline vein receives a tap from the left umbilical vein. The intrahepatic segment of this tap becomes the umbilical portion of the left portal vein. The flow in this segment reverses after birth and supplies segments of the left hemiliver. As the liver develops, the venous drainage of the parenchyma becomes focused on two vessels, the future right and left hepatic veins, and later the middle vein (not shown), which usually drains into the left hepatic vein. The ductus venosus develops as a through-channel from the left portal vein to the common hepatic vein. The remainder of the portal vein blood perfuses the sinusoids before reaching the hepatic veins. **C:** The vasculature is simplified with the removal of several segments including the most caudal anastomosis between the vitelline veins, the rostral portions of the left vitelline and left umbilical veins, and the right umbilical vein. The right lobe grows faster than the left because the left lobe loses the supply from the left vitelline vein and the left umbilical vein blood is shunted through the ductus venosus. The left umbilical vein actually lies in the midline and later shifts to the right of midline.

The liver occupies most of the abdominal cavity in the third month of gestation, in part because of large masses of sinusoidal hematopoietic cells. Thereafter, the right lobe grows faster than the left lobe but slower than the rest of the body. The liver cell cords remain tubular until birth, when they begin to remodel into double-cell plates and finally into single-cell plates by 5 years of age. Hematopoietic cells are still found in the sinusoids at birth and largely disappear from the liver by 4 weeks of age.

The hepatoblast is a bipotential progenitor cell that is positive for cytokeratin 19 (CK19) and HepPar1. During organogenesis, these cells differentiate into hepatocytes (CK19-negative and HepPar1-positive) and small bile ducts (CK19-positive and HepPar1-negative) (25,27). CK7 is expressed later and its level continues to increase in the weeks after birth. Severe injury to the adult liver causes a return to the pattern of expression seen in hepatoblasts. Therefore, regenerating epithelial cells in the liver have features of both ducts and hepatocytes (28).

Large Vessels of the Liver

The liver receives blood through the PV and through the HA, a branch of the celiac axis. Because the PV drains the blood of an area supplied by the other branches of the celiac axis and by the superior and the inferior mesenteric arteries, the hepatic blood flow depends on the flow in these arteries (29).

Portal Veins

The PV is an afferent nutrient vessel of the liver that carries blood from the entire capillary system of the digestive tract, spleen, pancreas, and gallbladder. It is constant in length, but the branches are variable (30,31) (Fig. 7.6). The PV is formed behind the neck of the pancreas by the confluence of the splenic and superior mesenteric veins. It also receives the superior pancreaticoduodenal vein, the left gastric (coronary) vein, and the cystic vein. Usually the upper 5 cm of the PV is devoid of major branches, allowing easy surgical dissection.

The splenic vein commences with five to six branches that return the blood from the spleen and unite to form a single nontortuous vessel. In its course across the posterior abdominal wall, it grooves the upper part of the pancreas, from which it collects numerous short tributaries. It runs close to the hilum of the left kidney and terminates behind the neck of the pancreas, where it joins the superior mesenteric vein at a right angle. Because of its nearness to the vessels of the left kidney, the splenic vein can be anastomosed to the renal vein. Its tributaries are the short gastric veins, the pancreatic veins, the left gastroepiploic vein, and the inferior mesenteric vein. In the distal splenorenal shunt operation, the short gastric veins are used for collateral drainage of the gastroesophageal varices.

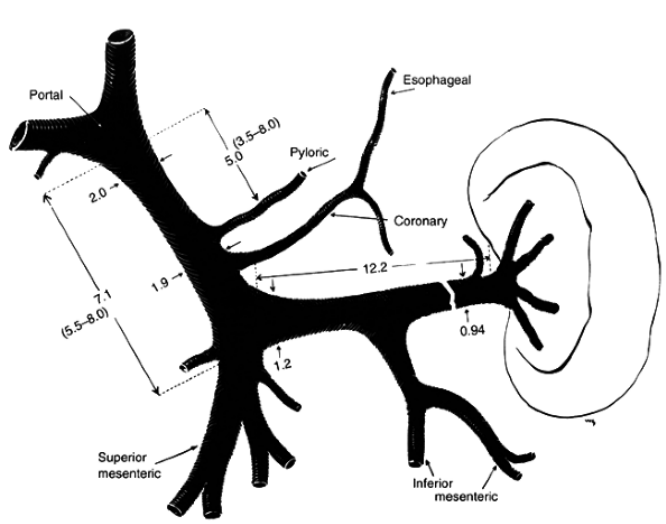
The superior mesenteric vein carries blood from the small intestine, ascending colon, and transverse colon. The inferior mesenteric vein returns blood from the area drained by the superior and the inferior left colic and the superior rectal veins.

An important tributary of the portal trunk is the coronary vein. It runs upward along the lesser curvature of the stomach, where it receives some esophageal veins. In patients with portal hypertension, these enlarge to form varices.

The portal trunk runs in the hepatoduodenal ligament in a plane dorsal to the bile duct and the HA and divides into two lobar veins before entering the portal fissure. The right lobar vein, short and thick, receives the cystic vein. The left lobar vein, longer and smaller, is joined by the umbilical vein and the paraumbilical veins. It connects with the inferior vena cava by the venous ligament. The left lobar vein gives branches to the quadrate lobe and also to the caudate lobe before entering the parenchyma at the left end of the porta hepatis. A separate branch may arise near the bifurcation to supply the caudate lobe. This vein is easily injured during dissection. The paraumbilical veins arise from the umbilical portion of the left PV and travel in the round ligament, where they may become evident as umbilical varices in the presence of portal hypertension. The umbilical vein is easily recanalized in infants, allowing access for blood sampling and angiographic visualization of the portal system.

In addition to the main PV and its branches, the liver receives other veins from the splanchnic circulation, the parabiliary venous system of Couinaud (32). This highly variable plexus includes several veins that arise from the pancreaticoduodenal or pyloric veins and drain into the PV or directly into the inferior surface

of segment IV and less often into other segments. This plexus provides examples of the metabolic effects of proximity to insulin source. Veins arising from the pancreatic region would carry blood with high insulin levels and pyloric veins would carry low-insulin-level blood. Because insulin determines the propensity of the liver to store triglycerides, the anatomy of these veins could explain some examples of focal fatty liver and focal fatty sparing (33).



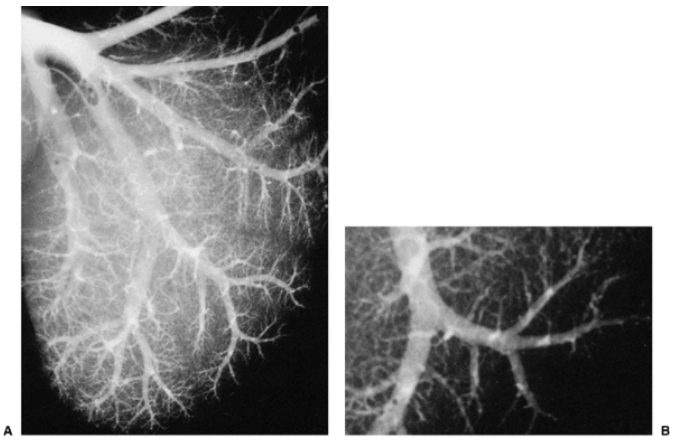
• **Figure 7.6** Measurements of the portal vein and its main branches, in centimeters. (From Gilfillan RS. Anatomic study of the portal vein and its main branches. *Arch Surg* 1950;61:449, with permission.)

Anomalies of the portal venous system are relatively rare. The anterior segment of the right liver is occasionally supplied by a branch of the proximal left PV (34). Preduodenal PV may be a result of the persistence of the most caudad anastomotic channel between the vitelline veins (Fig. 7.5). It may be associated with the duplication of the PV, annular pancreas, duodenal diaphragm, or intestinal malrotation, producing duodenal obstruction (35). Congenital absence of the PV is associated with portosystemic shunting because the superior mesenteric vein and the splenic vein drain directly to the vena cava or left renal vein, usually separately (36). This may be associated with hepatoblastoma or nodular hyperplasia of the liver (often simulating a neoplasm), cardiac anomalies, and biliary atresia. The ductus venosus rarely remains patent after infancy and is associated with hypoplasia of the intrahepatic PV branches, nodular hyperplasia of the liver, atrial septal defect, and hyperammonemia (37).

Atresia of the PV may be a congenital malformation often associated with other vascular anomalies or a response to neonatal injury such as omphalitis or PV thrombosis (38). When PV thrombosis occurs in the neonatal period, the vein does not grow so that in adulthood it appears as a thin fibrous cord (hypoplasia or agenesis). A thrombosed PV may develop numerous irregular intraluminal channels in addition to a leash of collaterals in the porta, giving a radiologic appearance called *cavernous transformation of the PV*.

Hepatic Veins

There are three main hepatic veins. The middle and left veins unite before entering the vena cava in 65% to 85% of individuals (7,39). In 18% of individuals, there are two right hepatic veins draining into the vena cava (20). In another 23%, there is a separate middle or inferior right hepatic vein draining segments V or VI, respectively. The veins have variable branching patterns. There are axial veins with four to six orders of dichotomous branching at acute angles, as well as numerous much smaller branches nearly at right angles (Fig. 7.7).



• **Figure 7.7 A:** Hepatic veins shown on postmortem angiogram. The major rami branch dichotomously and receive smaller branches nearly at right angles. **B:** Magnified portion of **(A)**.

The caudate lobe and adjacent parenchyma are usually drained by one or two small veins directly into the vena cava caudal to the main hepatic veins. When thrombosis of the main hepatic veins occurs, the veins of the caudate lobe are often spared, allowing survival and compensatory hyperplasia of this lobe (40). Anastomoses between branches of the hepatic veins are uncommon in the normal liver (41) but may be more frequent in the presence of diseases with portal hypertension (42). Anastomoses of veins to other lobes become enlarged and may be mistaken for the original hepatic veins on Doppler interrogation. Partial recanalization occurs, often leaving webs in the hepatic veins or vena cava. These webs were formerly thought to be congenital, although most are now considered to be acquired (43,44).

Hepatic Arteries

The common HA is the second major branch of the celiac axis (45). It courses to the right along the upper border of the pancreas in the right gastropancreatic fold, which conducts the artery to the medial border of the hepatoduodenal part of the lesser omentum. It ascends in front of the PV in 91% of humans and to the left of and behind the bile duct in 64% of cases. It gives off the left and the right HAs to supply the corresponding hemilivers. The right and left HAs each divide into two arteries that supply the right anterior and posterior sections and the left medial and lateral sections, respectively. The middle HA arises from the left or right HA and supplies the quadrate lobe.

The cystic artery arises from the right HA in the upper part of Calot's triangle (formed by the cystic duct, common hepatic duct, and inferior surface of liver). The cystic artery divides into a superficial branch that is distributed to the peritoneal surface of the gallbladder and a deep branch that supplies the attached wall of the gallbladder and adjacent liver. In 75% of cases, the artery is single, and in 25%, there are two arteries with separate origins for the deep and superficial branches.

Anomalies of the HA are frequent, occurring in half of the individuals (46). Angiographic studies of the HA demonstrate that the course of the arterial branches in the hilum deviates markedly from that of the PV, and in some cases the arteries may even cross the segmental fissures (47). However, the more distal arterial branches follow the PVs closely, "climbing along them like a vine on a tree" (48).

Anomalies of the HA have gained new importance because of the advent of transplantation, aggressive resections, and intra-arterial chemotherapy. The most important anomaly is a right HA arising from the superior mesenteric artery to supply the entire right liver (14%) (49). Because this vessel may appear in

Calot's triangle, it is at risk during cholecystectomy. The left HA arises from the left gastric artery in 14% to 25% of cases (45,49,50). This vessel enters the liver at the left end of the hilum and may fail to be ligated during resections, leading to hemorrhage. Each of the aberrant arteries may be the only HA so that its injury can damage the liver severely. The PV and HA to segment IV may cross to the left of the umbilical fissure before turning medially and, therefore, may be injured during left lateral segmentectomy. Ducts or small vessels cross this fissure in 20% of cases (13).

There are extensive communications between the ultimate and the penultimate branches of the right, the middle, and the left HAs in the umbilical fossa and in the region around the caudate lobe. The HA is provided with collateral flow through its anastomoses with arteries arising from the celiac axis and superior mesenteric artery. Anastomoses between the left and right HAs may occur. The main collaterals of the common HA are right and left gastrics; right and left gastroepiploics; gastroduodenal, supraduodenal, retroduodenal, superior and inferior pancreaticoduodenals; aberrant HAs; and inferior phrenic arteries (51).

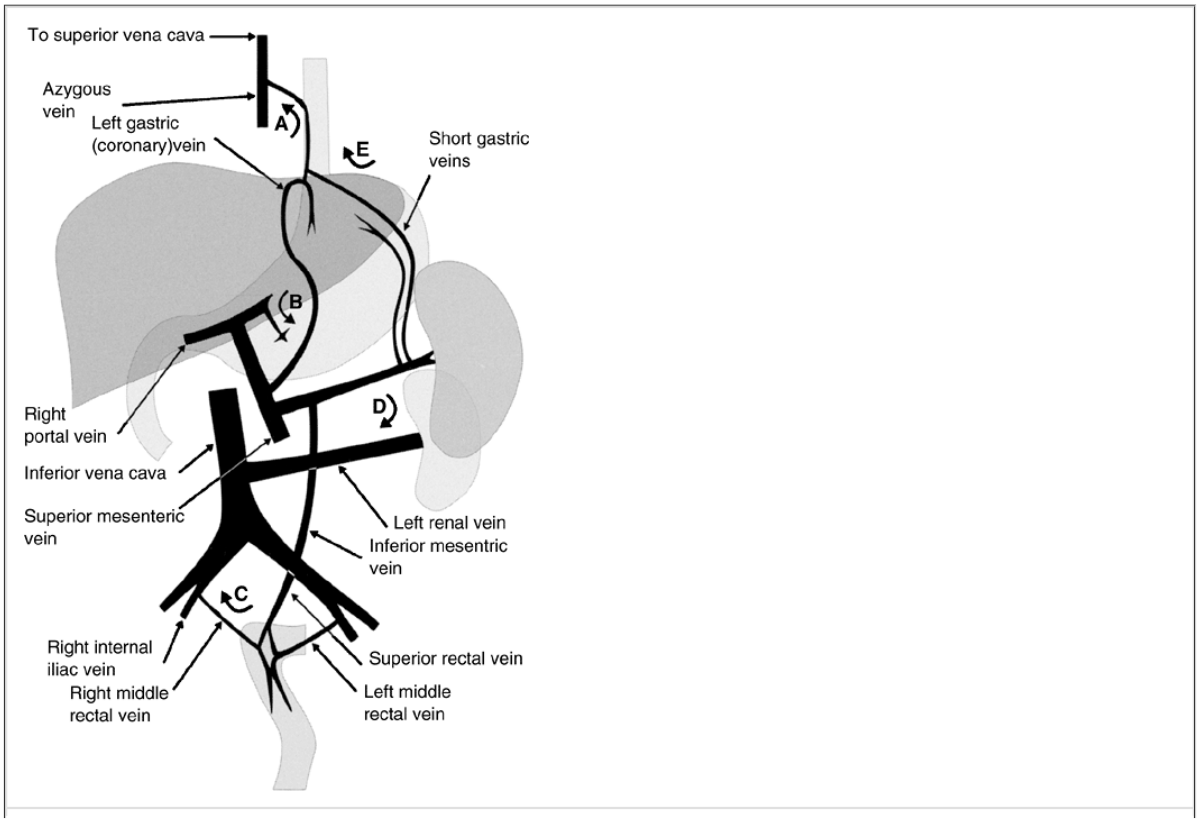
If ligation of an artery is necessary for the control of hemorrhage, ligation of the left or right artery is safe. Ligation of the HA proximal to potential collaterals such as the right gastric artery or gastroduodenal artery is better than ligation of the proper HA. After ligation of an HA, anastomotic channels enlarge and reestablish flow within a day (52).

Hepatic Collateral Circulation

Portal hypertension leads to the development of intra- and extrahepatic venous collaterals (53,54) (Fig. 7.8). Extrahepatic collaterals are important, because when dilated to form varices, they are susceptible to rupture and massive bleeding. Varices in the submucosa of the gastrointestinal tract are most often a problem, especially in the esophagus and stomach but also in the rectum and duodenum and at ostomy sites.

Dilated umbilical or paraumbilical veins are found in 11% of patients with cirrhosis (veins of Sappey) (55). They may cause a venous hum and *caput medusa* at the umbilicus (Cruveilhier-Baumgarten syndrome). Their presence implies high pressure in the left PV and, therefore, intrahepatic vascular obstruction. The direction of flow in lower abdominal wall collaterals is caudad if the inferior vena cava is obstructed, as in some patients with Budd-Chiari syndrome.

Varices may be found at sites where the gastrointestinal tract or pancreas becomes retroperitoneal or adherent to the abdominal wall because of pathologic processes. These "veins of Retzius" establish connections between the portal bed and the ascending lumbar azygos, renal, and adrenal veins.



• **Figure 7.8** Diagram of portal circulation. The most important sites for the potential development of portosystemic collaterals are shown. *A*, Esophageal submucosal veins, supplied by the left gastric vein and draining into the superior vena cava through the azygous vein. *B*, Paraumbilical veins, supplied by the umbilical portion of the left portal vein and draining into abdominal wall veins near the umbilicus. These veins may form a *caput medusa* at the umbilicus. *C*, Rectal submucosal veins, supplied by the inferior mesenteric vein through the superior rectal vein and draining into the internal iliac veins through the middle rectal veins. *D*, Splenorenal shunts: Created spontaneously or surgically. *E*, Short gastric veins communicate with the esophageal plexus. (From Wanless IR. Anatomy and developmental anomalies of the liver. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger and Fordtran's gastrointestinal and liver disease*, 6th ed. Philadelphia: WB Saunders, 1997:1057, with permission.)

Within cirrhotic parenchyma, shunts are formed by anastomoses between smaller branches of the portal and hepatic veins, as discussed later (56). These shunts allow blood to bypass the sinusoidal exchange surface, leading to functional impairment. This effect is made worse by the creation of large shunts. In addition, any procedure that decreases portal flow to the sinusoids increases the likelihood of thrombosis, further increasing intrahepatic resistance. Titration of these benefits and liabilities is an important feature of surgical management. Large spontaneous shunts may be beneficial in lowering portal pressure and should not be disturbed without due consideration.

Portosystemic shunting appears to be responsible for reduced peripheral vascular resistance, possibly through the enhanced release of nitric oxide (57). Dilated vascular channels in the lungs lead to intrapulmonary shunting with hypoxemia and increased cardiac output.

Lymphatics

Lymphatic channels are divided into deep and superficial networks (41,58). The former run parallel to the branches of the portal vessels and hepatic veins and the latter are found in the capsule. There are numerous anastomoses between these networks through small branches percolating through the capsule. The superficial lymphatics from the convexity form a dense network that coalesces into 14 groups of lymphatic trunks that drain through the coronary and falciform ligaments, through the diaphragm, and into esophageal and xiphosternal nodes. From the undersurface of the liver, they drain into hepatic hilar nodes. The deep lymphatics following the portal tracts reach the hepatic nodes at the left side of the porta hepatis, and the lymphatics along the hepatic veins drain to lymph nodes near the vena cava. The portal lymphatic trunks drain 80% of the hepatic lymph. The formation of lymph is discussed in the Section "Microanatomy."

Nerves

The liver has a rich sympathetic and parasympathetic innervation (59,60,61). Fibers derive from lower thoracic ganglia, the celiac plexus, the vagi, and the right phrenic nerve to form the plexi about the HA, PV, and bile duct. Most fibers are organized into the anterior and posterior trunks that enter the liver at the hilum. A few fibers enter at hepatic veins and ligaments. The arteries are innervated mainly by sympathetic fibers. The bile ducts are innervated by both sympathetic and parasympathetic fibers. Unmyelinated sympathetic fibers send branches to individual hepatocytes in zone 1. Nerve discharges are propagated from one hepatocyte to another through gap junctions (62).

Most hepatic nerve fibers are aminergic or peptidergic, with a few cholinergic fibers. Intra-acinar cholinergic fibers do occur in the guinea pig. Immunohistochemical studies have demonstrated many other substances in some hepatic nerve fibers, including vasoactive intestinal peptide, neuropeptide Y, glucagon, somatostatin, neurotensin, and calcitonin gene–related peptide. The effects of nerve stimulation are partially mediated by prostaglandins synthesized in nonparenchymal cells of the liver. There may be local baroreceptors capable of detecting sinusoidal hypertension and leading to reflex renal artery vasoconstriction (63,64). Afferent nerves may be responsible for pain when the liver is distended.

Stimulation of the nerve bundles around the HA and PV mainly results in a sympathetic discharge that alters the metabolism and hemodynamics of the liver. Glucose and lactate output are increased. Ketone and urea production, ammonia uptake, oxygen consumption, arterial and portal blood flow, and bile flow are reduced. Sympathetic nerve stimulation may exacerbate the effect of toxins (65). Parasympathetic stimulation is thought to increase glycogen synthesis and reduce glucose release. Hepatic parasympathetic activity has an important effect on skeletal muscle insulin resistance (66). Nerve action is modified by prevailing levels of hormones, especially insulin and glucagon. Resection of the anterior nervous plexus increases the concentration of bile salts and pigments in bile and impedes the accumulation of triglycerides in the liver. The clinical importance of this effect is uncertain. After transplantation, the denervated state of the liver persists (67), although hepatic blood flow and metabolic functions of the liver appear to be normal (68).

Biliary System

The biliary system includes the bile canaliculi, intrahepatic and extrahepatic bile ducts, peribiliary glands, gallbladder, and ampulla of Vater (27). The intrahepatic ducts begin at the bifurcation of the common hepatic duct.

Large Ducts and Gallbladder

The nomenclature of the large intrahepatic ducts will vary with the system used for naming the hepatic subunits (see preceding text). Each hepatic segment has a bile duct draining into a sectoral duct that drains into the right or left hepatic duct, which drains the right or left hemilivers, respectively. Caudate lobe drainage is variable, with ducts usually entering both right and left ducts. The junctions of the segmental, hepatic, and common hepatic ducts are also highly variable (69). The right and left hepatic ducts join to form the common hepatic duct at the right end of the portal fissure. The common hepatic duct is 1 to 5 cm long (mean 2 cm), is 0.4 to 1.3 cm in diameter (mean, 0.66), and is situated to the right of the HA and in front of the PV (70). It is joined by the cystic duct at its right side to form the common bile duct (ductus choledochus) that runs another 5 to 8 cm to the ampulla of Vater. The supraduodenal part of the common bile duct lies in the right border of the lesser omentum. The pancreatic part of the common bile duct passes retroperitoneally

behind the first portion of the duodenum. It then runs in a groove on the posterior surface of the head of the pancreas, anterior to the inferior vena cava. At the left side of the duodenum, it is joined in 70% to 85% of cases by the pancreatic duct (of Wirsung) and forms a common channel of variable length (71). When a dilatation is present, it is called the *ampulla of Vater* (72). The common channel resides within an elevation of the duodenal mucosa called the *major papilla* (of Vater).

The sphincter of Oddi consists of circular muscle fibers that surround the common bile duct in its course through the duodenal wall (73). Circular muscle fibers are also present around the end of the pancreatic duct and around the tip of papilla; longitudinal fibers are also present. The sphincter of Oddi is inhibited by cholecystokinin, assisting the expulsion of bile into the duodenum. An elongated common channel has been associated with congenital bile duct dilatation (74). Bile reflux may occur after papillotomy or surgical anastomoses with the intestine, resulting in recurrent cholangitis.

The gallbladder is a receptacle that receives up to a liter of bile daily, concentrating it by sodium-coupled water transport and expelling it on stimulation by cholecystokinin. The gallbladder is a pear-shaped sac with a volume of 30 to 70 mL and measuring 3 cm in width and 7 to 10 cm in length. Its parts are designated as fundus, body, and neck. It lies on the undersurface of the right liver lobe, with the fundus projecting beyond the inferior border of the liver where the lateral margin of the rectus crosses the costal margin. The body is

directed upward and to the left. Posteriorly, fundus and body are in close relation with transverse colon and duodenum, respectively. Gallstones can perforate into these viscera. The neck of the gallbladder is curved anteriorly and, when enlarged, forms the so-called Hartmann's pouch. The mucosa of the neck forms a spiral valve of Heister that continues into the cystic duct. The spiral valve has the function of regulating bile flow into and out of the gallbladder. The cystic duct measures 4 to 65 mm in length (mean, 30 mm) and 4 mm in average diameter (75).

The arterial supply of the bile ducts comes mainly from many branches of the common HA, especially the retroduodenal artery and the right HA (45,76). The gallbladder may be supplied by one, two, or three arteries. The cystic artery usually arises from the right HA. The veins of the gallbladder are variable, draining into the liver at the gallbladder bed or into the veins from the common bile duct, but eventually draining into branches of left and/or right PV (7). The lymph vessels of the gallbladder, hepatic ducts, and upper parts of the common bile duct empty into the lymph nodes of the hilum. Those of the lower common bile duct drain into nodes near the head of the pancreas.

Nerve fibers supplying the extrahepatic ducts and gallbladder derive mainly from the sympathetic hepatic plexus laced around the HA. These fibers also receive filaments from the right and left vagus nerves. Some nerve fibers deriving from the plexus can be seen running along the common bile duct. Sparse ganglion cells are present in the muscularis and the mucosa of the gallbladder. Their nervous connection with the spinal system is brought about by fibers from the right phrenic and musculophrenic nerves. Because these nerves derive from the third or fourth cervical nerve, the anatomic basis for shoulder pain in gallbladder disease is evident. Vagal stimulation causes gallbladder contraction (77).

Histology of the bile ducts and gallbladder has been reviewed by Frierson (78) and Nakanuma et al. (27). The wall of the extrahepatic ducts is formed by fibrous tissue with elastic fibers; smooth muscle is scanty or absent (79), except at the lower end of the common duct where muscle rings are conspicuous. The gallbladder wall contains abundant smooth muscle and little fibrous tissue. Rokitansky-Aschoff sinuses are outpouchings of the gallbladder mucosa through defects of the muscularis and are found in almost all gallbladders having calculi. The ducts of Luschka are small ducts in the areolar tissues of the hepatic surface of the gallbladder that communicate with intrahepatic bile ducts, but not usually with the gallbladder cavity, and may leak hepatic bile after cholecystectomy.

The mucosa has numerous papillary folds in the gallbladder, distal pancreatic duct, distal common bile duct, and ampulla. The mucosa of bile ducts and gallbladder consist of a single layer of columnar epithelium and a lamina propria. A few goblet cells are present, especially in the ampulla. Somatostatin-containing cells may be present in the ampulla, a possible source for the development of somatostatinomas arising at this site.

Mucous-secreting accessory glands (peribiliary glands) are in the lamina propria of the gallbladder neck and extrahepatic bile ducts and adjacent to the large intrahepatic ducts (80).

Intrahepatic Ducts

The intrahepatic ducts have been defined as ductules (<0.02 mm), interlobular ducts (0.02 to 0.1 mm), septal ducts (0.1 to 0.4 mm), and large ducts (>0.4 mm) (27). These measurements are approximate because the definition is also dependent on the relation to the segmental boundaries and on histologic pattern.

The large and septal bile ducts have a well-demarcated dense fibrous wall and high columnar epithelium with basal nuclei and small mucin droplets. These ducts express the blood group antigens. Interlobular ducts are located near the center of portal tracts and have minimal or no fibrous investment, and

the epithelium is low columnar or cuboidal and lacks mucin. There is a periodic acid-Schiff–positive basement membrane. The ductules are located near the limiting plate and have cuboidal epithelial cells. The presence of a few ductules may be considered normal, but large numbers are a feature of cholestatic or regenerating liver. Throughout the biliary tree, each duct is usually accompanied by an artery of similar diameter, a helpful guide when evaluating the absence of ducts.

The peribiliary glands accompanying intrahepatic large and septal ducts may be located within the walls (intramural) or form clusters outside the fibrous wall (extramural). Intramural glands are mucin rich, and extramural glands may be mucinous or serous, rarely with focal pancreatic acinar differentiation. Peribiliary glands are hypertrophied in patients with *Clonorchis* infestation and may be the tissue source of some cholangiocarcinomas (81). These glands may form retention cysts at the hilum in case of cirrhosis, PV obstruction, and polycystic kidney disease (82), rarely causing obstructive jaundice (83).

Intrahepatic bile ducts are the site of injury in many diseases, resulting in duct destruction, secondary cholestasis, and eventually, cirrhosis in severe cases. In primary biliary cirrhosis, ducts up to 0.3 mm in diameter are destroyed by an immune process. In primary sclerosing cholangitis, the most severely involved ducts are extrahepatic and large intrahepatic ducts, with less severe duct destruction in the smaller branches. Ducts less than 0.1 mm in diameter are the focus in chronic allograft rejection, graft versus host disease, Alagille's syndrome, and reactions to a variety of drugs and toxins. Neonatal biliary atresia, polycystic liver disease, and several other syndromes may be a result of a variety of insults to the developing ducts at the ductal plate stage (84).

The peribiliary vascular plexus is hypertrophied in livers with cirrhosis or with PV obstruction and is especially prominent in congenital hepatic fibrosis, where it may fill on portal venogram and appear to form a duplication of the portal tree (85).

Variations and Surgical Implications

A portion of the right liver may drain into the left duct system in 6% of cases (7). In 25% of cases, a branch of the right duct drains into the left duct (86). The common hepatic duct may receive accessory hepatic ducts. If the common hepatic duct is absent, the right and left hepatic ducts may run separately and join close to the duodenum; the right duct receives the cystic duct. Other variations include a main duct draining into the gallbladder, the cystic duct draining into the right hepatic duct, and the right hepatic duct draining into the cystic duct (7,69,85,87). The cystic duct usually enters the bile duct at an angle but may run parallel or curve behind the duct in a spiral manner. The relations of the large ducts and vessels near the hilum are variable, but the peripheral branches of these structures run together within portal tracts.

Because ducts depend on arterial supply, ischemic necrosis, with or without stricture, may occur in the large bile ducts after transplantation, especially if the HA is compromised. Duct strictures, rupture, and infarction of the gallbladder have also been found after hepatic arterial injection of alcohol or chemotherapeutic agents, possibly because of injury to the peribiliary vascular plexus (27).

Numerous conditions are characterized by congenital or acquired anomalies of the duct system. Aberrant biliary ducts, the vasa aberrantia, form anastomoses between the gallbladder and small ducts in the adjacent liver. These ducts are liable to leak bile after cholecystectomy. Accessory mucous-secreting periductal glands are located all along the duct system. These may develop retention cysts that, rarely, encroach on the duct lumen to produce obstructive jaundice (83). Congenital dilatation of the intrahepatic and/or extrahepatic ducts, known as *choledochal cyst*, is a rare cause of cholangitis or obstructive jaundice, usually presenting in childhood (see Chapter 43). Caroli's disease is a subset of this condition, with dominant dilatation of the intrahepatic ducts. Congenital fibrocystic disease occurs in a variety of anatomic patterns, often with coexistent renal disease. Clusters of dilated ducts within portal tracts, von Meyenburg complexes, are markers of adult polycystic kidney and polycystic liver diseases (88). Biliary atresia, the absence or obliteration of the extrahepatic bile ducts, is one of the most common causes of cirrhosis in childhood.

Anomalies and diseases of the gallbladder have been reviewed by Weedon (89). Absent gallbladder and double gallbladder are rare (0.05% and 0.02%, respectively) (90). Agenesis is associated with other congenital defects of the intestines or bones. Bilobed,

hourglass-constricted gallbladder and that with folded fundus (phrygian cap) or persistent septum favor retention and inflammation. Diverticulum also occurs. The gallbladder can be completely buried in liver substance or attached loosely to it by a mesentery (floating gallbladder).

Microanatomy

Normal Human Histology

When viewed histologically, the normal liver displays a uniform arrangement of portal tracts separated by parenchyma composed of hepatocellular plates and

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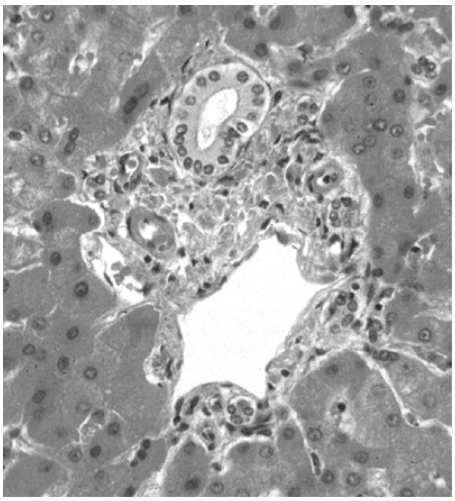
sinusoids. Terminal hepatic venules are located equidistant from portal tracts, which contain arteries, ducts, nerves, and PVs in a connective tissue stroma (Fig. 7.9). The stroma within portal tracts normally contains a small number of macrophages, plasma cells, and lymphocytes.



• **Figure 7.9** Normal portal tract from human liver, showing several small ducts, two arteries, a portal vein, and occasional lymphocytes (hematoxylin and eosin).

Although often called *portal triads*, the number of each of these elements varies with the size of the tract. In a study of needle biopsies, the number of profiles per portal tract was 2.3 ducts, 2.6 arteries, and 0.7 veins (91). The average minimum diameters were 13 μm for ducts, 12 μm for arteries, and 35 μm for PVs. In healthy infants, the number of ducts per portal tract is less than that in adults. The average number of portal tracts was 19 per biopsy, with a mean biopsy length of 1.8 cm. The interpretation of liver biopsies requires an assessment of sampling error. Biopsies obtained by the transjugular route are half this size, and in fibrotic conditions, the number of portal tracts available is often less than half a dozen.

Hepatocytes

Hepatocytes comprise 65% of the cells in the liver and 80% of hepatic volume. Hepatocytes are polyhedral with a central spherical nucleus. They are arranged in plates, one cell in thickness, with blood-filled sinusoids on each side of the plates (Fig. 7.10) (92). The cytoplasmic membrane has specialized domains providing a canalicular region on the lateral walls and numerous microvilli on the sinusoidal (basolateral) surfaces. The canalicular domains of adjacent hepatocytes are bound together by tight junctions to form bile canaliculi that coalesce and ultimately drain into ducts within portal tracts. Hepatocytes are also attached by gap junctions that have a role in the transmission of nerve impulses from zone 1 to zone 3. Normal and abnormal ultrastructure of hepatocytes has been reviewed elsewhere (90,93).

Endothelial Cells and Sinusoids

The length of a human sinusoid varies from 223 to 477 μm. The diameter of the sinusoids can vary from 6 to 30 μm and can increase to 180 μm when necessary. Zone 1 sinusoids are smaller than those in zone 3 (94). The caliber depends on active contraction of endothelial cells and stellate cells, as well as passive distension (95). Leukocytes are large compared to sinusoidal diameter, so that blood flow compresses the sinusoidal wall, promoting exchange between plasma, subendothelial fluid, and hepatocytes (94).

The sinusoidal surface is covered with a layer of endothelial cells that enclose the extravascular space of Disse (Fig. 7.10). Hepatic sinusoids differ from systemic capillaries in that the endothelial cells are fenestrated, subendothelial basement membrane material is scanty, Weibel-Palade bodies are absent in most species, and intercellular junctions are absent, permitting the passage of large macromolecules including lipoproteins but not chylomicrons (92,96). Fenestrations are grouped into clusters called *sieve plates*. Mean fenestration diameter in the rat is 150 to 175 nm, occupying 6% to 8% of the endothelial surface area (96). The fenestrations can change in size in response to various stimuli, including pressure, neural impulses, endotoxin, alcohol, serotonin, and nicotine (97). They are large in zone 1 and smaller and more numerous in zone 3. Agents that disrupt actin filaments can almost double the number of fenestrations within minutes (97). The permeability of fenestrations has been studied with marker particles. In the rat, liposomes 400 nm in diameter are readily engulfed by hepatocytes. The ability to traverse fenestrations may depend on the deformability or surface charge of the particles (98).

Sinusoidal endothelial cells also differ from continuous endothelial cells in their immunohistochemical phenotypes. Factor VIII–related antigen, *Ulex europaeus* agglutinin I binding, platelet/endothelial cell adhesion molecule-1 (PECAM-1), CD34, and 1F10 are features of continuous endothelial cells but not of sinusoidal endothelial cells (99). Sinu soidal endothelial cells express low-affinity Fc γ-receptors (CD32, FcR), lipopolysaccharide-binding protein complex receptors (CD14), thrombospondin receptors (CD36), class II histocompatibility receptors (CD4), and intercellular adhesion molecule-1 (100,101). During embryogenesis, the transition from

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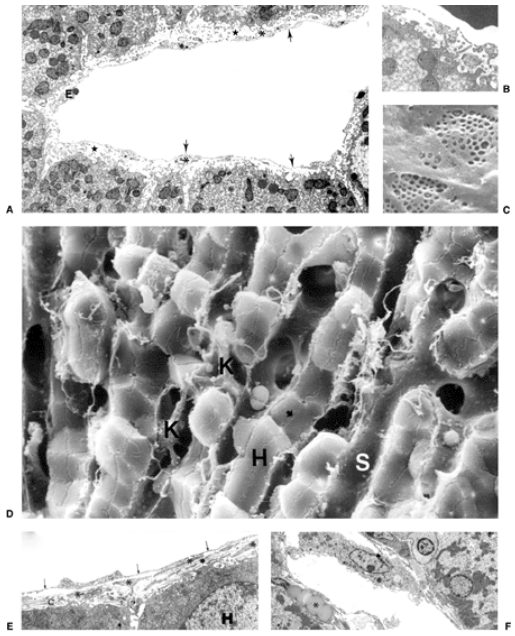
continuous to fenestrated phenotype occurs between 5 and 20 weeks' gestation (100). The fenestrated phenotype partially reverts to the continuous endothelial phenotype in chronic hepatitis, cirrhosis, and hepatocellular carcinoma (101), including the expression of CD34,

PECAM-1, and laminin receptors α6 β1 and α2 β1 (99).



• **Figure 7.10** Ultrastructure of sinusoids. (Courtesy of Dr. Bioulac-Sage.) **A:** Sinusoid showing endothelium covering the subendothelial space of Disse (*star*). This space contains stellate cell processes (*) and hepatocellular microvilli. Note that the microvilli extend into recesses between hepatocytes. The endothelial cells are fenestrated (*arrows*) (transmission electron microscopy [TEM] ×4,840). **B:** Closer view shows endothelial fenestrations and microvilli of a hepatocyte (TEM ×10,000). **C:** Endothelial fenestrations are clustered into sieve plates (scanning electron microscopy [SEM] ×29,400). (From Bioulac-Sage P, Saric J, Balabaud C. Microscopic anatomy of the intrahepatic circulatory system. In: Okuda K, Benhamou J-P, eds. *Portal hypertension: Clinical and physiological aspects.* Tokyo: Springer-Verlag, 1991:17, with permission.) **D:** Hepatocellular plates are one cell in width, with bile canaliculi (*arrow*) visible on the fractured edges of the plates. Hepatocytes (*H*), sinusoids (*S*), and Kupffer cells (*K*) are seen. Collagen fibers have been pulled from the spaces of Disse (SEM ×2,000). **E:** The space of Disse (*star*) contains several stellate cell processes (*) and collagen bundles (*C*). Endothelial fenestrations are labeled (*arrows*). *H*, hepatocyte nucleus (TEM ×6,000). **F:** Sinusoid with a Kupffer cell in the lumen and a stellate cell containing lipid (*) in the space of Disse (TEM × 2,000).

During the development of cirrhosis, the sinusoids also acquire some morphologic features of systemic capillaries; the space of Disse becomes widened with collagen, basement membrane material is deposited, endothelial fenestrations become smaller and less numerous, and hepatocellular microvilli are effaced. These changes, often called *capillarization of sinusoids* (102), likely reduce transport across the sinusoidal walls and explain some of the hepatocellular dysfunction seen in cirrhosis.

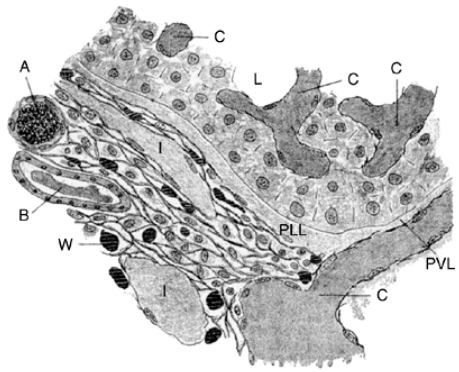
Nitric oxide produced by hepatocytes, Kupffer cells, and sinusoidal and arterial endothelial cells may cause increased sinusoidal blood flow, thereby protecting the liver during various injuries (103,104,105). Increased blood flow may be beneficial by preventing the adhesion of leukocytes and platelets that might otherwise injure the endothelium (60). Nitric oxide production may have a role in the hepatopulmonary syndrome (106) and hepatorenal syndrome (107). Endothelin-1 is produced by activated stellate cells and causes these cells to contract (108). Circulating endothelin-1 may have a role in hepatorenal syndrome (107).

Endothelial cell injury is important in endotoxemia, hypotensive shock, and cold perfusion of donor livers (109,110). Donor livers may develop rounding-up and detachment of endothelial cells that may be responsible for some instances of primary nonfunction after transplantation (111).

It has been suggested that thickening of the space of Disse may contribute to poor transport of materials to the hepatocellular surface (112), as well as possibly contributing to portal hypertension (113). Amyloid fibril deposition may widen the space of Disse dramatically and cause severe atrophy of subjacent hepatocytes. With severe amyloidosis, hepatomegaly, cholestasis, and noncirrhotic portal hypertension have been reported (114). Cellular infiltration within the lumina of sinusoids occurs in Gaucher's disease, mastocytosis, leukemias, and myeloproliferative disorders, but such infiltration does not correlate with the clinical evidence of portal hypertension (115). Obstruction of small veins is more likely to cause portal hypertension in these diseases because sinusoids are distensible and have the ability to regenerate (38).

Formation of Lymph

Most of the hepatic lymph derives from the subendothelial space of Disse, and a minority, perhaps 10%, is formed by leakage from capillaries of the peribiliary plexus. The smallest recognizable lymph capillaries are found in the interstitial tissue in terminal portal tracts and adjacent to terminal hepatic venules (116) (Fig. 7.11). The pathways that connect these lymph capillaries to the space of Disse have been difficult to demonstrate, and it is believed that lymph percolates through the collagen and proteoglycan matrix of the interstitium. Collagen bundles in the space of Disse appear to be continuous with fibers in the portal tracts, marking a submicroscopic channel for lymph flow. Lymph could also flow in the matrix investing the portal inlet venules and arterioles that penetrate the limiting plate.



• **Figure 7.11** Mall's drawing of portal and periportal tissue showing the space of Disse (perivascular lymph space, *PVL*), the space of Mall (perilobular lymph space, *PLL*), and lymph vessel (*I*) after injection with gelatin. The space of Disse is continuous with the space of Mall. In living beings, the space of Mall may be virtual where lymph percolates among interstitial matrix fibers. Also shown are lobule (*L*), sinusoids (*C*), connective tissue fibers (*W*), bile duct (*B*), and artery (*A*). (From Mall, FP. On the origin of the lymphatics in the liver. *Bull Johns Hopkins Hosp* 1901;12:146, with permission.)

Lymphatics have endothelial cells with no basement membrane and no pericytes. PVs can be distinguished from lymphatic channels by the presence of smooth muscle cells surrounding the former (117). The larger lymphatic vessels and trunks have valves.

Because of the large endothelial fenestrations in sinusoidal (and presumably lymphatic) endothelial cells, there is little or no oncotic pressure gradient between the plasma and the subendothelial tissue fluid, and the protein content of hepatic lymph is approximately 80% that of plasma. With a very low oncotic pressure gradient, the main stimulus for the formation of lymph is sinusoidal pressure. A 1-mm rise in efferent pressure doubles the hepatic lymph flow. The liver normally produces 1 to 3 L/day but this may increase to 11 L/day in cirrhosis or extrahepatic outflow obstruction (118). Communication between small bile ducts

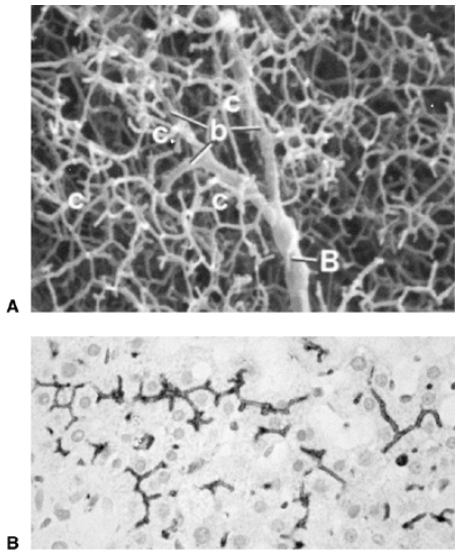
and lymphatics may allow for the increased formation of lymph seen after biliary tract obstruction (119).

Biliary Tree

The biliary tree begins with a network of bile canaliculi that empty into bile ducts (Fig. 7.12). Canaliculi have contractile and secretory properties.

Canaliculi in isolated hepatocyte doublets have been shown to undergo rhythmic contraction thought to represent peristaltic activity in the intact liver (120). These contractile functions are provided by a pericanalicular band of microfilaments composed of actin, myosin II, tropomyosin and α-actinin, and associated proteins, stabilized by a sheath of noncontractile intermediate filaments (121).

The cholangiocytes lining small ducts transport water and solutes under hormonal control (122,123). Peribiliary glands also participate in the concentration of bile. Chloride transport is dependent on the cystic fibrosis transmembrane conductance regulator, explaining the decreased water content of bile, hepatolithiasis, and secondary biliary cirrhosis seen in some patients with cystic fibrosis.



• **Figure 7.12** Bile canaliculi. **A:** Scanning electron micrograph of methacrylate injection cast of rat biliary tree (×860). *B*, terminal twig of the bile duct; *b*, canal of Hering; *c*, bile canaliculi emptying into canals of Hering. (From Murakami T, Itoshima T, Hitomi K, et al. A monomeric methyl and hydroxypropyl methacrylate injection medium and its utility in casting blood capillaries and liver bile canaliculi for scanning electron microscopy. *Arch Histol Jpn* 1984;47:223, with permission.) **B:** Photomicrograph of human liver with slight cholestasis, stained for carcinoembryonic antigen (CEA). CEA is present in the distribution of the bile canaliculi that could not be seen on hematoxylin and eosin.

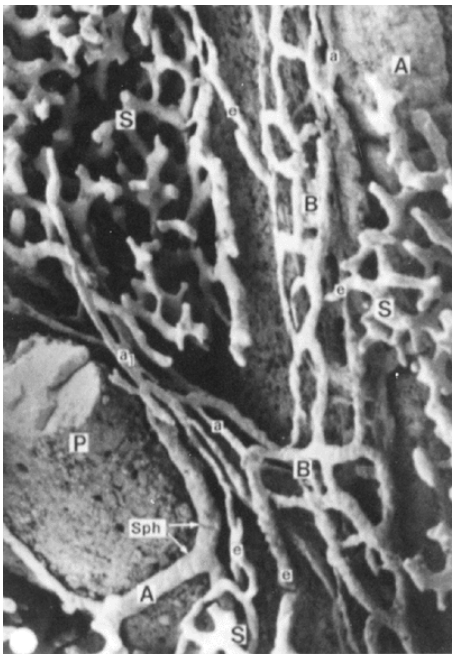
The small bile ducts are supplied by arteries (124) (Fig. 7.13). Terminal branches of the HA supply a general capillary plexus within the

portal tract and a peribiliary vascular plexus and also empty directly into zone 1 sinusoids (125). The general and peribiliary vascular plexi eventually drain into sinusoids through capillary connections known as the *internal roots of the PV*. The peribiliary vascular plexus has been divided into inner, intermediate, and outer layers. The inner layer has fenestrated endothelium, suggesting a role in water exchange. Similar fenestrated endothelium is found in the gallbladder mucosa.

Stellate Cells

The stellate cells (fat-storing cells or Ito cells) are located within the spaces of Disse. Their cytoplasmic droplets normally contain abundant vitamin A, mostly

as retinyl palmitate (126). These cells can be identified by the presence of immunoreactivity for smooth muscle actin, transient autofluorescence, and histochemical affinity for gold and silver. When activated by various cytokines, stellate cells are transformed into myofibroblast-like cells that have reduced vitamin A storage, increased content of myofilaments and α-smooth muscle actin, and increased expression of procollagen gene transcripts. Stellate cells, in their activated state, are the principal hepatic fibroblasts (127,128). Evidence that many forms of hepatic injury activate hepatic macrophages and that these cells release cytokines capable of activating stellate cells has been presented (129). Stellate cells can also secrete matrix metalloproteinases that degrade matrix proteins (130). Activated stellate cells contract under stimulation by sympathetic discharge or endothelin-1 secretion (95,108,131). By this mechanism, stellate cells could exacerbate portal hypertension.



• **Figure 7.13** Scanning electron micrograph of cast blood vessels in the liver of rhesus monkey. Peribiliary arterial plexus (*B*) receives blood from arterial branches (*A*) by means of afferent arterioles (*a*). The plexus supplies sinusoids (*S*) through efferent arterioles (*e*). Note the grooves indicating arteriolar sphincters (*Sph*). Arterioles (a_1) bypass the plexus and empty directly into sinusoids. *P*, portal vein (methyl methacrylate cast ×135). (Courtesy of Dr. T. Murakami, Department of Anatomy, Okayama University, Japan.)

Kupffer Cells

Kupffer cells are the resident macrophages in the liver. They comprise more than 80% of tissue macrophages in the body and 15% of cells in the liver. Although capable of proliferation in situ, they are also recruited from the peripheral blood (127). Kupffer cells reside in the sinusoids, with pseudopodia anchored to endothelial cells or, occasionally, hepatocytes. They may form part of the sinusoidal wall.

Liver-Associated Lymphocytes

A few lymphocytes are normally found in portal tracts and sinusoids even after washout with saline. Portal lymphocytes are 90% T cells with CD4/CD8 ratio of 1.6, whereas sinusoidal lymphocytes are 60% T cells with a CD4/CD8 ratio of 0.4 and 30% natural killer cells (CD56+). Sinusoidal lymphocytes reside in the lumen adherent to Kupffer cells and endothelial cells (127). Many sinusoidal lymphocytes are large, with cytoplasmic granules, and are also called *pit cells* because of the resemblance of the granules to grape seeds (128). Pit cells are most numerous in zone 1 sinusoids. These cells are thought to have a role in killing tumor cells and virus-infected cells. The granules of pit cells contain perforin, a protein that injures cell membranes. Pit cell tumoricidal activity is enhanced by the presence of Kupffer cells (128).

Stroma

Connective tissue stroma supports the capsule, the portal tracts from hilum to periphery, and the sinusoidal walls. The composition of the stroma varies with location. Connective tissue of the capsule and portal tracts is mostly type I and III collagen and elastin. Reticulin fibers, defined by their histochemical affinity for silver, are largely composed of type III collagen and fibronectin (132). They are located in the spaces of Disse, where they give tensile strength to the parenchyma. Type IV collagen forms a basal lamina around small vessels.

Many noncollagenous glycoproteins are present in the matrix, including laminin, fibronectin, tenascin, entactin, vitronectin, undulin, osteonectin, and Von Willebrand's factor (133). Laminin links basement membrane collagen to the integrins attached to endothelial cells and epithelial cells. The function of tenascin is uncertain, but it is mitogenic for a variety of cell types. Vitronectin stimulates fibroblast migration; von Willebrand's factor is found within endothelial Weibel-Palade bodies and in basement membranes (133). Proteoglycans bind to cells and matrix proteins and have roles in matrix–cell and cell–cell interaction.

In scarred livers, there is an absolute increase in many matrix proteins. The bulk of the scar tissue is type I collagen. After

hepatocellular necrosis, the connective tissue framework is rapidly repopulated with hepatocytes in an orderly manner. If regeneration is delayed, the deposition of collagen by stellate cells destroys the framework and prevents restitution to normal.

The stromal collagen is important in the prevention of tears in the blood vessels and sinusoidal walls. Focal dissolution of reticulin leads to parenchymal hemorrhage and blood-filled cysts (peliosis hepatis) (134,135). Mineral oil deposits are present in portal tracts and adjacent to hepatic venules in more than half of human livers and are usually accompanied by a slight mononuclear infiltrate (136).

Three-Dimensional Organization of the Liver

The organization of the hepatic parenchyma has been conceptualized in two contrasting models: The acinus and the lobule (137,138,139). Terminal PVs interdigitate with the terminal hepatic venules, with sinusoids bridging the gaps between these vessels. The terminal hepatic venules can be considered as being in the center of a lobule or the periphery of several acini. The acinar approach is discussed in detail here.

The simple liver acinus is a small parenchymal mass, irregular in size and shape, arranged around an axis consisting of a terminal hepatic arteriole, portal venule, bile ductule, lymph vessels, and nerves that grow out together from a small portal field (Fig. 7.14). The simple liver acinus lies between two (or more) terminal hepatic venules with which its vascular and biliary axis interdigitates. In a two-dimensional view, it occupies sectors of two adjacent hexagonal or pentagonal fields.

The plates and cords of the simple acini are in continuity with adjacent acini in three dimensions; there

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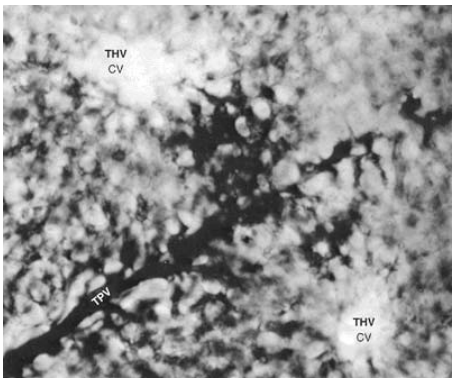
is no capsule separating the acini from one another. It can be assumed that the dividing line between the acini is the watershed of biliary drainage, so that each acinus empties its biliary secretion into the axial bile ductule.



• **Figure 7.14** Liver acinus in humans. The acinus occupies sectors only of two adjacent hexagonal fields and reaches their central veins (*CV*). The terminal portal branch (*TPV*) is injected with India ink and runs perpendicular to the two terminal hepatic venules (*THV*) with which it interdigitates (thick cleared section ×300).

A complex acinus is a clump of tissue composed of at least three simple acini whose axial channels branch in three dimensions from the preterminal stalk (Fig. 7.15). Each of its terminal branches forms the axis of a simple acinus. A sleeve of tissue composed of tiny clumps (acinuli) surrounds the preterminal channels. These acinuli are nourished by axial venules and arterioles branching off the preterminal vessels. Structural and functional unity in a complex acinus can be demonstrated by injecting colored materials (140). The

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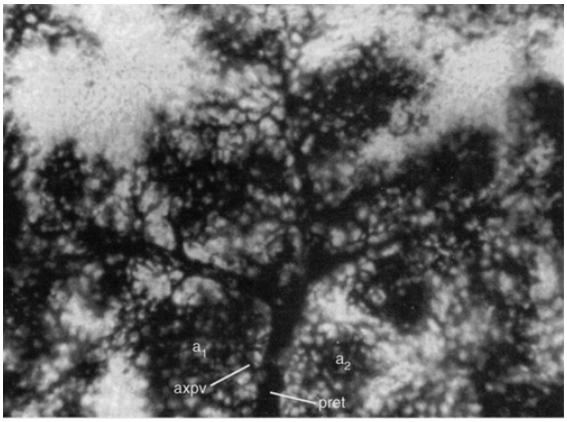
axial vessels of the simple acini are always the same color as the parent vessels of the complex acinus. Three or four complex acini form larger clumps of tissue, the acinar agglomerates. Acini or acinuli also form a sleeve of parenchyma around the axial stem servicing the agglomerate.



• **Figure 7.15** Complex acinus in humans. The sinusoids injected with India ink are supplied by three terminal portal branches and their parent preterminal vessel (*pret*). These portal venules help form the axial channels of a complex acinus cut longitudinally. The sleeve of parenchyma around *pret* is formed by acinuli (a_1, a_2); *axpv*, axial portal venule supplying the sinusoids of a_1. The poorly injected *white* areas (in the upper corners) are parts of zone 3 around terminal hepatic venules, which are not shown (150 µm-thick section ×88).

• **Figure 7.16** Group of acinar agglomerates in a human liver injected with India ink. Three large portal branches grow out in different directions from a portal space (*PS*). One of these runs diagonally through the field and represents the axis of an acinar agglomerate. From this portal branch, preterminal (*1*) and terminal (*2*) branches grow out and form the axes of complex and simple acini, respectively (100 μm-thick cleared section ×18).

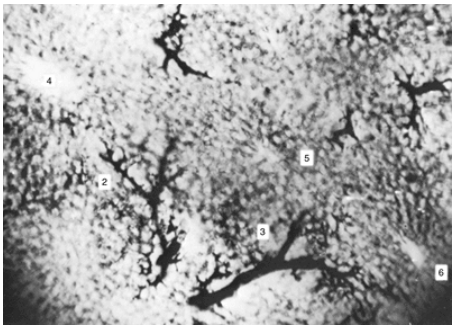
The acinar agglomerate has unity because the main route of vascular supply and the biliary drainage are common to the whole clump, as well as to its subdivisions. The hierarchy can be continued because several agglomerates are supplied by a single macroscopic portal tract. The PVs supplying agglomerates in the human liver are approximately 150 μm in diameter (140) (Fig. 7.16). All branches of the hepatic vein interdigitate with HA and PV branches of similar order; this creates a hexagonal or pentagonal pattern when seen histologically in cross-section (Figs. 7.17 and 7.18).

The acinus is an ideal physiologic unit for understanding many vascular and biliary events in human biology (see discussion on cirrhosis in subsequent text). The conceptual advantage of the acinus is that the blood supply to a portion of the parenchyma and the bile duct draining the same parenchyma reside in the same portal triad. "Thus, structural, circulatory, and functional unity is established in this small clump of parenchyma" (141) (Fig. 7.19). By contrast, the classical hexagonal lobule is supplied by several separate PV branches, arteries, and ducts, each of which also supplies other adjacent lobules (140).

McCuskey (142) notes that all the essential relationships are present within smaller units called *hepatic microvascular subunits*. This concept is validated by the existence of focal degenerative changes that involve portions of parenchyma that are subacinar in size. The smallest useful unit is that in which there is a significant barrier to blood flow between adjacent units.

Recent studies show that PV blood is distributed by numerous small inlet venules, giving a portal supply that is more diffuse and less granular than that pictured by the original description of the acinus (137,138,139). These analyses suggest that isobars of oxygen tension are sickle shaped (Fig. 7.20), with the parenchymal subunits being components of a hedge rather than individual grapes on a vine. Although parenchymal subunits are difficult to visualize in the normal human liver, they become evident in pathologic conditions such as nodular regenerative hyperplasia, in which there is a pruning of the portal venous supply. As with a hedge, when individual portal units are pruned, the remaining units undergo hyperplasia to form an

array of spherical units, revealing the underlying acinar structure. The hierarchical arrangement of simple, complex, and agglomerate acini can be appreciated in livers with prominent atrophy or necrosis (141). In this century-long debate, it is useful to note that parenchymal subunits do not exist in the human liver. The debate concerns the best way to imagine their structure if they did exist.



• **Figure 7.17** Interdigitation of portal and hepatic vein branches. Human liver injected with India ink. Two horizontal terminal portal branches (*2, 3*), forming the axes of acini, interdigitate with three vertical terminal hepatic venules (*4, 5, 6*), around which they arch (cleared thick section × 110).

Hepatocellular Heterogeneity

The liver is anatomically situated to receive high concentrations of nutrients and certain hormones from the intestines and pancreas. Gradients of these substances, as well as oxygen and waste products, are found across the functional units of the liver. These gradients are not constant but vary with cycles of feeding and exercise.

The position of hepatocytes within the acinus is reflected by the specialized functions of these hepatocytes (138,143,144,145) (Fig. 7.21). Zone 1 hepatocytes are adapted to high oxidative activities, having numerous large mitochondria. Dominant processes in zone 1 are gluconeogenesis, β-oxidation of fatty acids, amino acid catabolism and ureagenesis, cholesterol synthesis, and bile acid secretion. Zone 3 is an ideal location for exergonic processes, including glycolysis and lipogenesis. There is a narrow rim of hepatocytes adjacent to terminal hepatic venules that remove ammonia from the blood by synthesizing glutamine. Zone 3 is also the site of general detoxification and biotransformation of drugs.

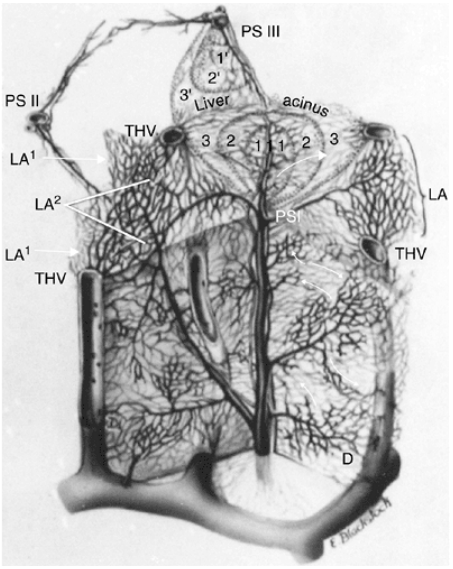
Metabolic zonation is accompanied by gradients of some anatomic features (141). Mitochondria are larger and more numerous and lysosomes and Golgi are more abundant in zone 1. Smooth endoplasmic reticulum is more abundant and nuclear volumes are larger in

zone 3. Endothelial fenestrae are larger and less numerous in zone 1. Kupffer cells, large granular lymphocytes, stellate cells, and sympathetic nerve endings are all more numerous in zone 1. There are also gradients in the composition of matrix proteins (146).

The control of metabolic zonation has been found to operate at the pretranslational level, with a few exceptions. Therefore the gradients of the enzyme protein and the enzyme messenger ribonucleic acid tend to parallel each other. Enzyme expression usually varies during the feeding cycle or changes in oxygen tension, and the zonation is said to be dynamic. It is likely that many signals interact to produce dynamic zonation. Glucose and oxygen gradients each have an effect on various enzymes, and these gradients are interdependent (147,148). The sensors of oxygen gradients may be mediated by heme-containing proteins.

Stable zonation that does not vary with metabolic signals is a result of intercellular or cell–matrix interactions. For example, glutamine synthetase activity

may depend on close approximation of hepatocytes with some element of the venules, although this is controversial (144,149). Metabolic zonation is lost in cirrhosis (150). Glutamine synthetase expression was undetectable in one study (150).

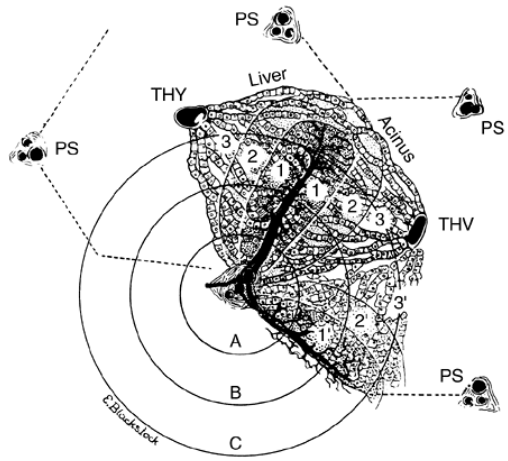


• **Figure 7.18** Vascular and biliary architecture of an acinar agglomerate and the relation of the acini to the adjacent hexagonal lobule. Note the arcuate courses of the terminal portal branches, the irregularly arranged simple acini, and the short portal vessels that form the axes of tiny acinuli constituting the mantle of parenchyma around the longitudinally cut portal space. Intercommunicating paths of acini and acinuli are indicated by *arrows*. *PS I, PS II, PS III*, portal spaces; *LA, LA*^{1}, simple liver acinus; *LA*^{2}, simple acinus penetrating a hexagonal field situated well above the level of origin of the acinus; *THV*, terminal hepatic venule; *D*, channels of Deysach; *1, 2, 3*, circulatory zones of the simple liver acinus.

Although hepatocellular heterogeneity is largely determined by the zonal gradients of environment cues, some heterogeneity occurs because of local genetic aberration. This can be seen in hemochromatosis as iron-free foci within livers that are otherwise diffusely pigmented (151). These represent dysplastic foci with increased risk for the development of hepatocellular carcinoma (152). Clusters of hepatocytes with excess cytoplasmic glycogen occur in patients with various disorders of ureagenesis and in leprechaunism, presumably because of focal genetic variation (153).

Clinical Importance of Hepatocellular Heterogeneity

Metabolic heterogeneity is responsible for zonal injuries that are of diagnostic value to the pathologist. The distribution of necrosis and steatosis in response to chemical injury is often zonal (154). Sharply defined zone 3 necrosis is characteristic of toxicity due to acetaminophen, *Amanita phalloides*, pyrrolizidine alkaloids, and various hydrocarbons such as halothane and carbon tetrachloride. Zone 1 necrosis has been found with allyl alcohol, phosphorus, and high-dose iron ingestion. Zone 2 toxicity is rare in humans but has been produced in animals with ngaione, furosemide, and beryllium. Cocaine toxicity in rodents may affect different zones depending on preexisting enzyme induction (155).

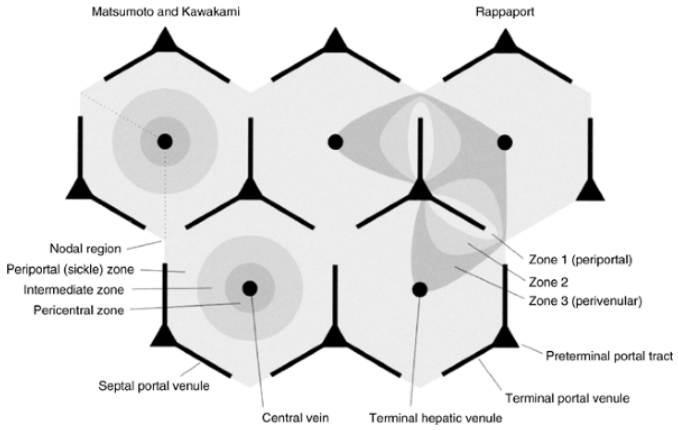


• **Figure 7.19** Blood supply of the simple liver acinus and the zonal arrangement of cells. The acinus occupies adjacent sectors of neighboring hexagonal fields. Zones *1, 2*, and *3*, respectively, represent areas supplied with blood of first, second, and third quality, with regard to substrate, oxygen, and nutrients. These zones center on the terminal afferent vascular branches, terminal bile ductules, lymph vessels, and nerves and extend into the triangular portal field from which these branches crop out. Zones *1′, 2′*, and *3′* designate corresponding areas in a portion of an adjacent acinar unit. In zones *1* and *1′*, portal inlet venules empty into sinusoids. Note that zone *3* approaches the preterminal portal tract, nearly reaching the inner circle (*A*). *PS*, portal space; *THV*, terminal hepatic venules (central veins).

Systemic hypoperfusion generally produces zone 3 necrosis. This pattern may be altered by local factors. For example, in disseminated intravascular coagulation, zone 1 sinusoidal fibrin deposition results in maximum ischemia being located in zone 1 or 2. Zone 2 necrosis has been reported in some patients with hypotensive shock (156). Viral hepatitis usually produces spotty necrosis in all zones but often with an ill-defined zone 3 predominance. Yellow fever often produces zone 2 necrosis. Herpesvirus produces well-demarcated necrosis that does not follow a zonal distribution.

In hemochromatosis, hemosiderin is deposited predominantly in zone 1 hepatocytes. This is useful to

distinguish hemosiderin from lipofuscin, the latter occurring predominantly in zone 3 hepatocytes. Proximity to a source of insulin favors the development of steatosis, as seen after the instillation of insulin in the peritoneal cavity during dialysis and in the case of decreased steatosis in infarcts of Zahn, where PV supply to the tissue is obstructed.



• **Figure 7.20** The acinar structure of the hepatic microcirculation, as conceived by Rappaport (140) and modified by Matsumoto and Kawakami (137). In both models, the margins of the shaded zones represent planes of equal blood pressure (isobars), oxygen content, or other characteristic. The models differ in the shape of the isobars surrounding terminal portal venules. The acinus is bulb-shaped, and the classical hexagonal lobule is comprised of several wedge-shaped portions (called *primary lobules*, indicated by *dotted lines*, upper left), which have cylindrical (sickle-shaped) isobars. The nodal region is the nodal point of Mall (22). (From Wanless IR. Anatomy and developmental anomalies of the liver. In: Feldman M, Scharschmidt BF, Sleisenger MH, eds. *Sleisenger and Fordtran's gastrointestinal and liver disease*, 6th ed. Philadelphia: WB Saunders, 1997:1059, with permission.)

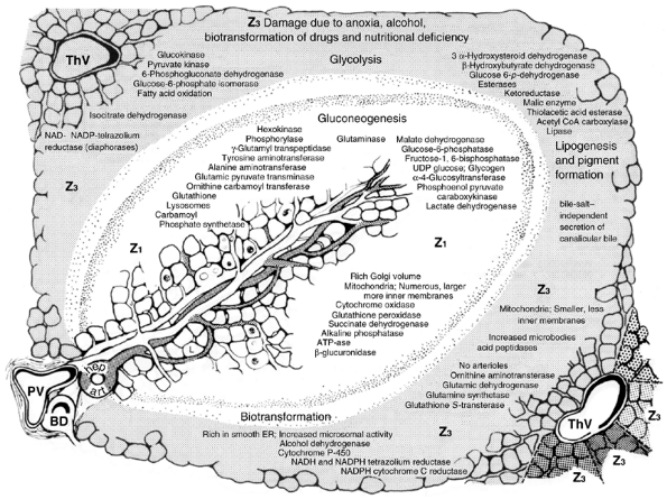
Control of Hepatic Microcirculation

The terminal PVs supply sinusoids directly, giving a constant but sluggish blood flow (140,141). In contrast, arterioles drain into terminal portal venules and zone 1 sinusoids, giving a pulsatile but small volume flow that appears to enhance sinusoidal flow, especially in periods of reactive arterial flow, as in the postprandial state. Groups of sinusoids shift their work asynchronously. The change from the storage phase of inactive sinusoids to flow activity demonstrates, at the microscopic level, the function of the liver as a "venesector and blood giver of the circulatory system" (157).

Arterial flow varies inversely with PV flow. The mechanism of this *hepatic arterial buffer response* is based on the washout of locally produced adenosine (158). When portal venous flow is reduced, adenosine accumulates and causes dilatation of the arterial resistance vessels; the reverse also occurs. The relative contribution of arterial and portal venous flow varies between regions of the liver and with

gravity and other physiologic variables (159). Conductance of PVs increases with distention, causing portal pressure to be little altered by large changes in PV flow (160). In addition to arteriolar tone, local control of the microcirculation may depend on the contraction state of sinusoidal endothelial and stellate cells (95).

Regional blood flow is of practical importance when investigating focal lesions such as focal nodular hyperplasia and neoplasms. The venous and arterial circulation can be differentially imaged by using computed tomography with arterial portography (CTAP) or CT scan with intravenous contrast injection. Hepatocellular carcinoma, hepatic adenomas, metastases, and focal nodular hyperplasia are mostly supplied by arteries and are therefore hypodense with CTAP (161,162). Cirrhotic nodules, dysplastic nodules, and small hepatocellular carcinomas may have a portal venous supply and are usually isodense on CTAP.



• **Figure 7.21** Schematic diagram of various metabolic processes that show zonal differences across the acinus. PV, portal vein; *ThV*, terminal hepatic venule; *D*, bile duct; *A*, hepatic arteriole; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; UDP, uridine diphosphate; ER, endoplasmic reticulum; Z_1, periportal area; Z_3, periacinar and perivenular area. (The latter is derived from portions of zone 3 of several adjacent acini.) (Modified from Rappaport AM, Wanless IR. Physioanatomic considerations. In: Schiff L, Schiff ER, eds. *Diseases of the liver*, 7th ed. Philadelphia: JB Lippincott, 1993:1–41, Jungermann K, Kietzmann T. Zonation of parenchymal and nonparenchymal metabolism in liver. *Annu Rev Nutr* 1996;16:179–203.) (For description see text; for references see both the sources.)

Physioanatomic Aspects of Human Liver Disease

Regeneration of Hepatocytes

The normal liver maintains a constant mass that is determined by the needs of the host. If a partial hepatectomy is performed, the organ grows to regain much of the original mass in 10 days in rodents and in a few weeks or months in humans (163). If a liver is transplanted into a new host, the new liver grows (by mitosis) or shrinks (by apoptosis) to the size of liver expected for the host's body size. Hepatocyte dysfunction, such as chronic cholestasis in primary biliary cirrhosis or primary sclerosing cholangitis, appears to be a signal to initiate cell proliferation because the liver may be twice the normal weight in the early stages of these diseases.

There is controversy about the source of the hepatocytes participating in regeneration. The normal resting liver has a very low mitotic rate. After many types of injury, mitoses become readily visible in hepatocytes from all acinar zones. However, some labeling studies suggested that mitosis is particularly active in the periportal region, giving rise to the hypothesis that hepatocytes are born in zone 1 and migrate in their lifetimes to zone 3 (164). This "streaming liver" concept implies the presence of stem cells, now considered to be located in the smallest radicles of the biliary tree, the canals of Hering (165,166). Cells in this location are thought to be able to differentiate into cholangiocytes and hepatocytes (167,168). It appears that the bulk of cell replacement, in acute and subacute injury models, is by panacinar mitosis. The stem cell and streaming liver mechanisms may be important in severe injury states (169). The stem cell population may be replenished by cells migrating from the bone marrow (170,171,172,173,174).

In a healthy human liver, half the hepatocytes are diploid and the other half are polyploid (175). One third of the hepatocytes are binuclear. The processes of polyploidization and binucleation are irreversible, so that polyploidy increases with age. These processes may protect long-lived hepatocytes against mutation. During a regenerative stimulus, binucleation and polyploidy decrease as binucleating growth is suppressed. Therefore, after regeneration, freshly divided cells have small and uniform nuclei, in contrast to the polyploid resting cells.

Putative signals for the initiation of the cell cycle in hepatocytes include hepatocyte growth factor (HGF), produced in all the major types of nonparenchymal liver cells; epithelial growth factor, produced in the salivary glands and hepatocytes; transforming growth factor-α (TGF-α), produced in the hepatocytes, and tumor necrosis factor (TNF) (see Chapter 2) (176). Endotoxin stimulates TNF secretion, and TNF stimulates Kupffer cells to secrete interleukin-6, which is an important mediator of hepatocellular regeneration. HGF and TGF-β are bound to the extracellular matrix (177). Insulin and glucagon support hepatocyte growth in culture and in vivo, although they are not complete mitogens for hepatocytes. Although the details of this complex system are not fully understood, it is clear that cytokines and growth factors derived from multiple cells types are involved. Therefore, gut-derived endotoxin, pancreatic hormones, activated Kupffer cells, and sinusoidal blood flow will influence hepatocellular regeneration (171).

Table 7.2. Clinical and Anatomic Features of the Major Forms of Chronic Liver Disease

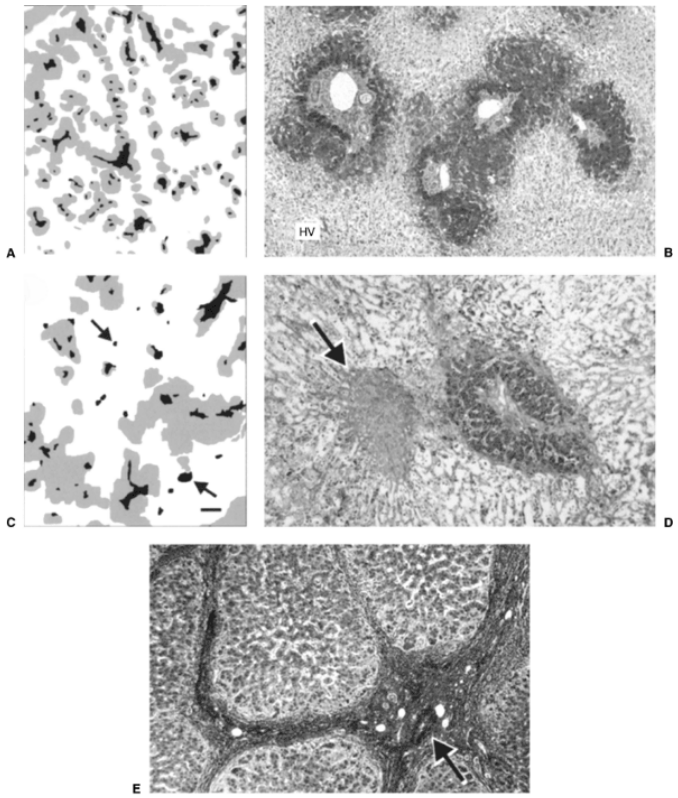
Anatomic	Portal	Hepatocellular	Parenchymal	Fibrous	Obliteration of small	Extrahepatic or large intrahepatic	Obliteration of small hepatic

diagnosis	hypertension	dysfunction	extinction	septa	portal veins	PV block	veins
Cirrhosis, micronodular	++	++	+++	Many, broad	+++	±	+++
Cirrhosis, macronodular	+	±	++	Many, narrow	++	±	++
Cirrhosis, venocentric[a]	++	+	+++	Between hepatic veins, portal tracts not involved	+	±	+++
Cirrhosis, incomplete septal	±	±	+	Moderate, narrow, or incomplete	+	-	+
Cirrhosis, incomplete septal with large portal vein obstruction	+	±	+	Moderate, narrow, or incomplete	+	+	+
Extrahepatic portal vein obstruction[b]	++	±	-	None	+	++	-
Nodular regenerative hyperplasia	±	±	-	None or rare	++	±	±

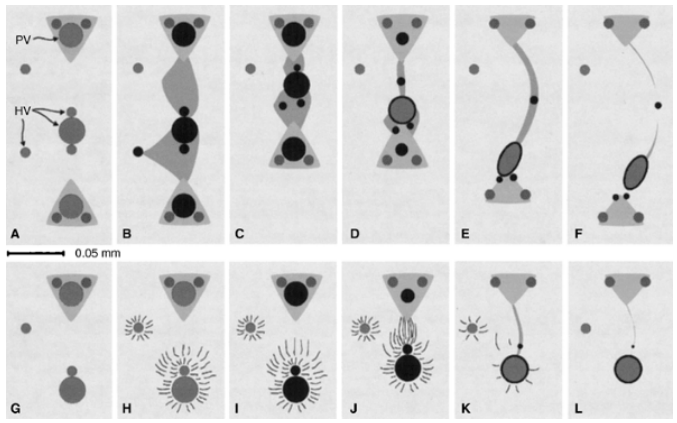
Gradings are for typical examples but gradings can vary within each anatomic category.
[a]Typically seen with Budd-Chiari syndrome.
[b]Examples are Portal vein thrombosis, portal vein agenesis, cavernous transformation of the portal vein.

Pathogenesis of Chronic Liver Disease

Chronic liver disease is usually defined as hepatic injury lasting for at least 6 months. The end stage of chronic liver disease has often been divided in two clinicopathologic forms: Cirrhosis and noncirrhotic portal hypertension, the former being associated with portal hypertension and hepatic dysfunction and the latter with portal hypertension and nearly normal function. Recent anatomic studies have demonstrated that these two categories can be further subdivided (Table 7.2) (38). All types of chronic liver disease are associated with vascular obstruction, and the various anatomic patterns can be explained by the distribution and severity of the obstructive lesions. Each anatomic pattern is the summation of numerous local parenchymal lesions of either atrophy or extinction. Therefore, understanding the pathogenesis of atrophy and extinction is necessary to understand the pathogenesis of each anatomic pattern.



• **Figure 7.22** The histogenesis of cirrhosis. There are two basic types of cirrhosis, venocentric and venoportal. The type of cirrhosis is determined by the distribution of vascular obstruction, as illustrated by these examples with Budd-Chiari syndrome. **A, B:** Venocentric cirrhosis occurs when parenchyma near the obstructed hepatic veins (*HV*) shows contiguous cell loss and fibrosis (extinction) and cirrhotic nodules (*dark tissue*) contain portal tracts with patent veins. The periportal tissue survives because of retrograde portal vein drainage. **C, D:** When hepatic vein obstruction is followed by portal vein thrombosis, retrograde portal vein (PV) flow is not possible and the periportal tissue cannot support hepatocytes. This is shown in **(C)** where portal tracts (*black*) are usually accompanied by periportal tissue (*gray*) except in regions affected by PV thrombosis (*arrows*). In **(D)** the *arrow* indicates a portal tract with obstructed PV and absent periportal tissue. **E:** Venoportal cirrhosis occurs when portal tracts are incorporated into the septa located adjacent to the cirrhotic nodules. Arrow shows obliterated portal vein (**B**, **D**, and **E**: Elastic-trichrome stain). (Modified from Tanaka M, Wanless IR. Pathology of the liver in Budd-Chiari syndrome: Portal vein thrombosis and the histogenesis of venocentric cirrhosis, venoportal cirrhosis, and large regenerative nodules. *Hepatology* 1998;27:488–496, with permission.)



• **Figure 7.23** Diagrammatic depiction of tissue remodeling in chronic hepatitis **(A–F)** and in alcoholic liver disease **(G–L)** during the development and regression of cirrhosis. Normal acini are shown in **(A)** and **(G)**, with the sequence of events leading to small regions of parenchymal extinction in the following panels. Obstructed veins are shown as *black circles*. **B:** Obliteration of small portal and hepatic veins occurs early in the development of cirrhosis in response to local inflammatory damage. The supplied parenchyma becomes ischemic. **C, D:** Ischemic parenchyma shrinks and is replaced by fibrosis (process of extinction). The shrinkage is accompanied by close approximation of adjacent vascular structures. **E:** Septa are deformed and stretched by expansion of regenerating hepatocytes. **F:** As septa are resorbed, they become delicate and perforated before disappearing. Trapped portal structures and hepatic veins are released from the septa and are recognizable as deformed and ectopic remnants. Note the absence of portal veins. In alcoholic disease **G–L**, the sequence of events may differ from other forms of chronic liver disease. **H:** Sinusoidal fibrosis is often prominent before parenchymal collapse, leading to a pericellular pattern of fibrosis. **I, J:** Inflammation and fibrosis lead to hepatic and portal vein obliteration, with secondary condensation of preformed sinusoidal collagen fibers into a septum. **K, L:** After prolonged periods of inactivity, sinusoidal fibrosis and septa are resorbed. (Modified from Wanless IR, Nakashima E,

Sherman M. Regression of human cirrhosis. Morphologic features and the genesis of incomplete septal cirrhosis. *Arch Pathol Lab Med* 2000;124:1599–1607, with permission.)

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The two-hit vascular model allows one to predict the local parenchymal response to vascular obstruction (178,179). Normal liver parenchyma is supplied by arteries and PVs and is drained by hepatic veins. The dual blood supply, therefore, allows for two levels of ischemia. Mild ischemia may occur when any one of these vessels is obstructed, resulting in atrophy. Severe ischemia occurs when any two of these vessels are obstructed, leading to extinction. The evolution of extinction lesions is illustrated in Figure 7.22.

The histologic appearance of the liver varies with time after injury, with progressive enlargement (nodular hyperplasia) of well-supplied regions of parenchyma, arterial dilatation and growth, and progressive resorption of collagen (178,180). These secondary events complete the genesis of the various patterns of chronic liver disease. Therefore, nodular hyperplasia occurring in the presence of atrophy produces the pattern of nodular regenerative hyperplasia. Nodular hyperplasia occurring in the presence of abundant extinction is the pattern called *cirrhosis*. The pattern of cirrhosis is further modified by the relative involvement of portal and hepatic veins (Fig. 7.23). Resorption of collagen in cirrhotic livers leads to macronodular cirrhosis, incomplete septal cirrhosis, and eventually almost a total regression of cirrhosis (Fig. 7.24). Significant regression presupposes that the activity of disease and the development of new obstructive events are minimized by spontaneous remission or effective therapy.

The mechanism of vascular obstruction in chronic liver disease depends on the nature of the primary disease. In most forms of chronic hepatitis, portal and hepatic vein phlebitis occurs as a bystander effect of inflammation in adjacent tissue (181). In Budd-Chiari syndrome and chronic congestive failure, thrombosis is the cause of the vascular obliteration (179). In chronic

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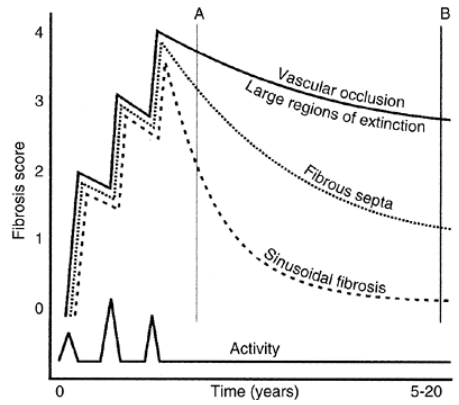
biliary disease, bile salt injury is likely responsible for the hepatic vein obliteration, and portal inflammation may explain the PV obliteration. In established cirrhosis of any etiology, stasis commonly leads to secondary thrombosis or congestive venopathy of portal and hepatic veins and secondary parenchymal extinction that may be independent of the activity of the original disease (182,183). The significance of these thrombotic and congestive venopathy mechanisms is that they point to mechanisms that might be ameliorated by therapy. Antithrombotic therapy is often recommended in patients with obvious thrombotic disease such as Budd-Chiari syndrome and portal thrombosis (184) but may also be beneficial in other types of progressive liver disease. Congestive venopathy might be ameliorated by β-blockers or porta-caval or transhepatic shunt procedures.



• **Figure 7.24** The time course of the histologic features of cirrhosis in relation to periods of activity. After cessation of activity, various histologic features regress at different rates. The balance of histologic features varies with the duration of low-activity disease, for example, between time points *A* and *B*. At time *B*, cirrhosis cannot be diagnosed histologically, although portal hypertension may remain. (Reprinted with permission, from Wanless IR. Regression of human cirrhosis. In Reply. *Arch Pathol Lab Med* 2000;124:1592–1593.)

The development of extinction in chronic hepatitis may be recognized as bridging necrosis in very active cases. In less active disease, there are clusters of apoptotic and atrophic hepatocytes, often with congestion of the sinusoids. As lesions are fully organized, they are identified by the approximation of hepatic veins to portal tracts after the intervening parenchyma has been resorbed.

The formation of intrahepatic shunts in cirrhosis can be understood from this model. Arterioles from terminal portal tracts normally feed into zone 1 sinusoids. When the local terminal hepatic venule is obliterated, arterial flow seeks a patent channel with lower pressure. If the local PV is patent, it receives the arterial flow by retrograde flow in the zone 1 sinusoids (179). If the local PV is also obstructed, the arterial flow will drain laterally into those sinusoids that connect with a patent hepatic vein, likely the parent of the obstructed terminal hepatic venule. These favored sinusoids dilate and become part of an artery-to-hepatic vein shunt. If the nearest patent hepatic vein is too distant, the parenchyma becomes congested and eventually extinct.

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Chapter 8
Bilirubin Metabolism and Jaundice

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Key Concepts

- Bilirubin is the final degradation product of heme, which is the prosthetic group of numerous important hemoproteins involved in oxygen transport (e.g., hemoglobin) or metabolism (e.g., P-450 cytochromes). The conversion of heme to bilirubin involves two enzymatic steps: Opening of the heme ring by heme oxygenase to form biliverdin, with the release of both carbon monoxide (CO) and iron, and reduction of biliverdin to bilirubin by biliverdin reductase.
- Healthy adults produce a mean of 4 mg of bilirubin/kg body weight per day. Degradation in the reticuloendothelial system of the hemoglobin of dying erythrocytes generates 80% to 85% of daily bilirubin production. The remainder has multiple sources, including ineffective erythropoiesis in the bone marrow and the turnover of short-lived, nonhemoglobin hemoproteins including the various P-450 cytochromes. Because synthesis and degradation of these cytochromes occurs throughout the body, both heme biosynthesis and the heme oxygenase/biliverdin reductase pathway are widely distributed.
- Bilirubin was long considered simply a biologic waste product. However, biliverdin and bilirubin have antioxidant properties, CO is important in cell signaling, iron plays a key role in the generation of reactive oxygen species, and both heme biosynthesis and heme degradation are tightly regulated. These observations suggest that, hemoglobin degradation aside, heme synthesis and degradation throughout the body may play a role in cellular antioxidant defenses.
- Bilirubin formed in the periphery is kept in solution during transit to the liver by very tight binding to albumin. Once in the liver, it is transported from plasma to bile by four distinct steps: Hepatocellular uptake; binding to specific intracellular proteins; conversion to a water-soluble form by conjugation to glucuronic acids by the uridine-5′-diphosphate glucuronosyltransferase isoform UGT1A1; and the adenosine triphosphate (ATP)-dependent, carrier-mediated transport of the resultant bilirubin monoglucuronide (BMG) and bilirubin diglucuronide (BDG) across the canalicular domain of the plasma membrane into the bile canaliculus.
- Jaundice is the yellow–orange discoloration of the skin, conjunctivae, and mucous membranes that results from an elevated concentration of bilirubin in plasma. Hyperbilirubinemias are usually classified into those that are predominantly unconjugated and those that are mainly conjugated. The latter often, in fact, involve elevations of levels of both the conjugated and unconjugated bilirubin fractions. Instances of hyperbilirubinemia in which other common hepatic biochemical test results are normal often reflect familial hyperbilirubinemias; the combination of hyperbilirubinemia with abnormalities of other hepatic biochemical tests suggests an acquired condition. Exceptions to this rule are not rare.
- The plasma unconjugated bilirubin concentration reflects a balance between bilirubin turnover and hepatic bilirubin clearance (C_{BR}). Unconjugated hyperbilirubinemia can result from increased bilirubin turnover (e.g., hemolysis), decreased bilirubin clearance (e.g., neonatal hepatic immaturity, familial unconjugated hyperbilirubinemias), or situations in which both processes are occurring (neonatal immaturity in infants with glucose-6-phosphate dehydrogenase deficiency).
- Reduced levels of UGT1A1 on either a congenital (Gilbert's and Crigler-Najjar syndromes) or an acquired basis (administration of certain human immunodeficiency virus protease inhibitors) and shunting of blood around the hepatic parenchyma in cirrhosis are the most frequent causes of a reduction in C_{BR}. Hereditary (Dubin-Johnson syndrome) or acquired (hepatocyte injury) deficiencies of the canalicular transport system, or obstruction to the flow of bile down the biliary tract, are the principal causes of conjugated hyperbilirubinemia.
- Application of molecular technology has led to extensive progress in understanding the pathogenesis of the familial hyperbilirubinemias. Gilbert's syndrome and Crigler-Najjar syndrome types I and II were long considered separate diseases, with separate patterns of inheritance. It is now recognized that they all reflect autosomal recessive disorders characterized by mutations of different severity in the gene encoding UGT1A1. Similarly, Dubin-Johnson syndrome is now known to reflect an inherited abnormality in multidrug resistance–associated protein 2 (*MRP2*), encoding an ATP-dependent canalicular plasma membrane transporter for bilirubin conjugates and a number of other non–bile acid organic anions. Of the five familial hyperbilirubinemias, only Rotor's syndrome remains unexplained in terms of molecular pathogenesis.

Bilirubin, a reddish-yellow heme degradation product, is produced principally by the breakdown of the hemoglobin of senescent red blood cells and eliminated from the circulation by the liver. Jaundice, derived from the French *jaune* (yellow), is the yellow–orange discoloration of the skin, conjunctivae, and mucous membranes, which is a consequence of an elevated concentration of bilirubin in plasma. Although mild hyperbilirubinemia may be clinically undetectable, jaundice becomes apparent at plasma bilirubin concentrations of 3 to 4 mg/dL. The threshold for its recognition depends on the patient's normal pigmentation, the lighting conditions under which the observation is made, and the particular fraction of plasma bilirubin whose concentration is elevated. Optimal interpretation of an elevated plasma bilirubin concentration is based on an appreciation of its metabolism, and in particular, its sources and disposition. These are the subjects of this chapter.

Studies of bilirubin chemistry, metabolism, and genetic disorders underwent explosive growth in the 1960s through the 1990s, creating the foundation on which current work in the field is based. Because space limitations restrict the number of references that can be cited in this chapter, readers are frequently referred to review articles for detailed bibliographies of older work in this field (1,2,3,4,5,6,7,8,9,10).

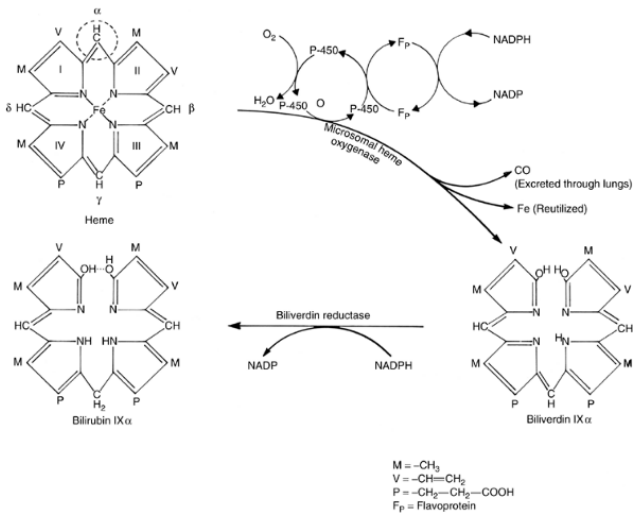
Sources, Structure, and Plasma Transport of Bilirubin

Bilirubin Production from Heme

Bilirubin is the final, common end product of the metabolism of heme, the moiety found in hemoglobin, myoglobin, and other hemoproteins (Fig. 8.1). The formation of bilirubin is the result of a multistep, enzymatic process in which the porphyrin ring of heme is first opened at the α-bridge carbon in a stereoselective, enzymatic oxidative process carried out by the microsomal enzyme heme oxygenase. This step leads to the release of an iron atom and the formation of equimolar quantities of biliverdin, a green tetrapyrrolic pigment, and carbon monoxide (CO) (3,4,11,12,13,14,15). Biliverdin is a water-soluble pigment readily excreted unaltered by the liver. It is the principal bile pigment in many

amphibia, fish, and birds. However, it does not readily cross the placenta. Accordingly, most mammals rapidly and quantitatively convert biliverdin to the reddish yellow pigment bilirubin through a reaction that is catalyzed by the enzyme biliverdin reductase (2,3,8,9,16). Heme oxygenase is present in macrophages throughout the reticuloendothelial system, including Kupffer cells of the liver, and certain epithelial cells, including hepatocytes and renal tubular cells (17). Biliverdin reductase is widely distributed in many cells throughout the body, including macrophages (16). The major sites of bilirubin production are the spleen and other compartments of the reticuloendothelial system, which degrade the hemoglobin of senescent and injured red blood cells. However, the degradation of heme to bilirubin can occur in many sites, including macrophages that migrate into hematomas containing extravasated hemoglobin. Because both heme oxygenase and biliverdin reductase are present in macrophages, the sequential steps in the conversion of heme to bilirubin are readily visualized at the edges of any bruise, where the purplish to green to yellow color changes reflect the conversion of extravasated and deoxygenated hemoglobin first to biliverdin and then to

bilirubin (18,19).

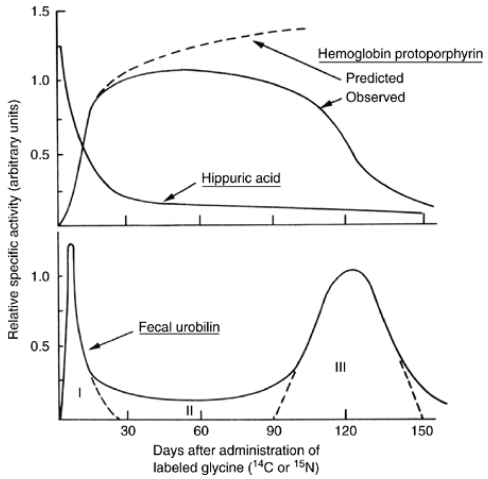


▪ **Figure 8.1** Pathway for the degradation of heme to bilirubin. Stereospecific opening of the heme macrocycle at the α-bridge carbon by the microsomal enzyme heme oxygenase results in the formation of equimolar amount of biliverdin and carbon monoxide. Biliverdin is subsequently reduced to bilirubin by the enzyme biliverdin reductase. MET, microsomal electron transport system; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form). (Reproduced from reference Berk PD, Jones EA, Howe RB. Disorders of bilirubin metabolism. In: Bondy PK, Rosenberg LE, eds. *Metabolic control and disease*, 8th ed. Philadelphia: WB Saunders, 1979:1009–1088, with the permission of the publisher.)

Possible Cytoprotective Effects of the Heme Oxygenase/Biliverdin Reductase Pathway

Although both heme oxygenase and biliverdin reductase were initially considered to function solely as a heme-degradative and waste disposal pathway, the

widespread distribution of these enzymes in cells outside the reticuloendothelial system, the tight control of heme oxygenase activity achieved through the presence of both an inducible (HO-1) and a constitutive (HO-2) form of the enzyme, and the important biologic effects of the several products of heme degradation have led to a growing interest in this pathway (20). In this regard, both biliverdin and bilirubin have proved to be potent antioxidants, CO functions as both a signaling molecule and an important vasoactive regulator, and the iron released during heme degradation contributes to various forms of cellular cytotoxicity by facilitating the formation of reactive oxygen species (ROS). These findings suggest that cells may have evolved a fine control over the heme oxygenase/biliverdin reductase pathway specifically to regulate CO production for signaling and heme consumption and generation of bilirubin and biliverdin for their roles in counteracting intracellular oxidative and nitrosative stress (20,21). The existence of a specific oxidation/reduction cycle in which lipophilic ROS oxidize bilirubin to biliverdin, which is then re-reduced by biliverdin reductase, has been postulated (22) and debated (23). Such a cycle, analogous to the glutathione/glutathione disulfide (GSH/GSSG) cycle for detoxifying soluble oxidants, would permit bilirubin to destroy a 10,000-fold excess oxidants (22).



▪ **Figure 8.2** Relative specific activities of hemoglobin protoporphyrin, fecal urobilin (stercobilin), and hippuric acid after administration of labeled glycine. The early labeled peak of stercobilin is derived from ineffective erythropoiesis and the turnover of heme enzymes; the late peak reflects the death of senescent erythrocytes. The observed specific activity of hemoglobin protoporphyrin is less than that predicted from the continued availability of labeled glycine for hemoglobin synthesis, as determined from the hippuric acid curve. This suggests some random loss of labeled erythrocytes, which may be the source of fraction II of labeled stercobilin. (Reproduced from reference Berk PD, Jones EA, Howe RB. Disorders of bilirubin metabolism. In: Bondy PK, Rosenberg LE, eds. *Metabolic control and disease*, 8th ed. Philadelphia: WB Saunders, 1979:1009–1088, with permission of the publisher.)

Quantitative Aspects of Bilirubin Production

In healthy human subjects, bilirubin production averages approximately 4 mg/kg body weight per day (6 μmoles/kg body weight per day)

(2,5,8,9,14,15,24,25). Hemoglobin from senescent or injured erythrocytes is the source of 80% to 85% of the heme that is eventually catabolized to bilirubin (Fig. 8.2). The remainder has multiple sources, including a component of ineffective hemoglobin production in the bone marrow and the turnover of short-lived, nonhemoglobin hemoproteins such as the various P-450 and b_5 cytochromes, catalase, and peroxidase (1,2,3,8,9,10,25,26,27,28,29,30,31). In normoblastic hemolytic anemias, the bone marrow can increase red blood cell production by as much as eightfold (32), leading to a corresponding increase in the component of bilirubin production derived from erythrocytes (3,5,27). Under these conditions, the amount of bilirubin derived from ineffective erythropoiesis in the marrow may increase

in absolute terms, but the proportion of bilirubin production due to ineffective erythropoiesis remains unchanged (27,28). By contrast, major increases in the fraction of bilirubin production derived from ineffective erythropoiesis may occur in megaloblastic anemias such as those associated with either folate or vitamin B_{12} deficiency, thalassemia, and certain dyserythropoietic anemias (27,28). Increased bilirubin production also occurs in the disorders of heme biosynthesis, including the hereditary erythropoietic porphyrias and lead poisoning (27,28,29). Finally, administration of phenobarbital and other drugs increases the turnover of heme enzymes, notably hepatic cytochrome P-450 isoforms, with a resulting increase in bilirubin production (30,31,33) (see following text).

Structure of Bilirubin-IXα

Naturally occurring bilirubin is designated bilirubin-4*Z*,15*Z*-IXα. This designation indicates that it is derived from protoporphyrin isomer IX, the isomer found in heme and hemoproteins such as hemoglobin, by cleavage of the porphyrin macrocycle at the α-bridge carbon and that the stereochemical arrangement of the 4,5 and 15,16 double bonds places the 5- and 15-bridge carbons is in the *Z* configuration (12,34) (Fig. 8.3). This configuration allows the formation of internal hydrogen bonds between the propionic acid side chain on the B ring and polar groups on the D ring, and between the propionic acid on the C ring and polar groups on the A ring (35). Although bilirubin is frequently depicted as a linear tetrapyrrole, these hydrogen bonds in fact fix the molecule in a "ridge tile" configuration (34,36). This configuration blocks exposure of the molecule's polar groups to aqueous solvents and of the central bridge carbon to attack by diazo reagents and is therefore the basis for both bilirubin's hydrophobic behavior and its slow (indirect) diazo reactivity (reviewed in ref. 12,37) (see following text).

Other Bilirubin Isomers

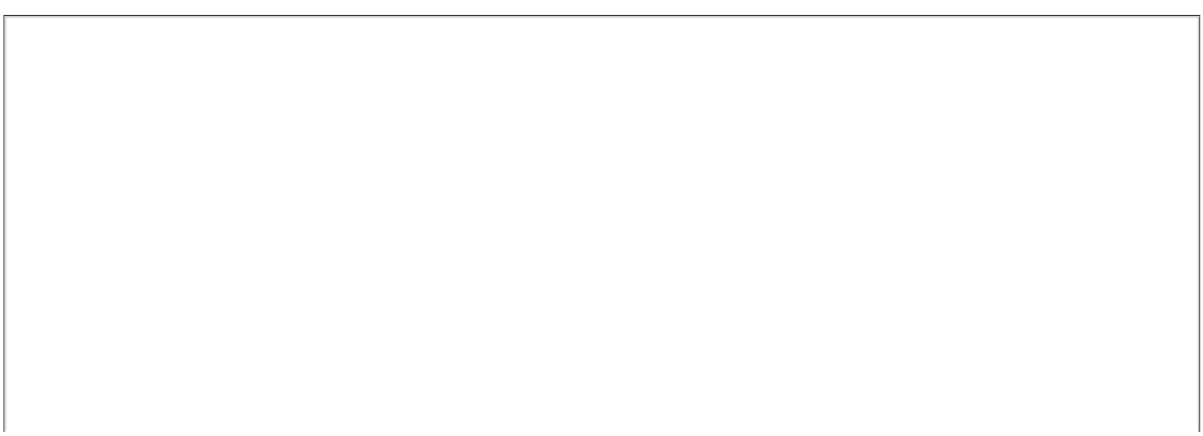
A number of structural (Fig. 8.4A and D) and configurational (stereo-) isomers (Fig. 8.4B and C) of bilirubin are of either physiologic or clinical interest. Opening of the protoporphyrin-IX ring at bridges other than the α-carbon can occur nonenzymatically, leading to the formation, after reduction, of bilirubin-IXβ, IXγ, or IXδ (Fig. 8.4A) (34,38). Stereoisomerization at positions 4 and 15 can lead to the formation of 4*Z*,15*E* and 4*E*,15*E* stereoisomers, respectively (12,34,39), as illustrated in Figure 8.4C and D. None of these isomers can form the internal hydrogen bonds characteristic of bilirubin-4*Z*, 15*Z*-IXα. Accordingly, they behave as more polar molecules, with rapid (direct) diazo reactivity, and can be excreted in bile without conjugation (40,41,42,43). Finally, under certain conditions in vitro, the two nonidentical halves of the bilirubin molecule can dissociate and then reassemble at random (4,12,39). This results in the formation of two symmetric isomers, designated bilirubin-IIIα and XIIIα, in addition to the asymmetric IXα isomer; the ratio of the IIIα:IXα:XIIIα molecules formed is approximately 1:2:1 (Fig. 8.4B). Bilirubin-IIIα and XIIIα *can* form internal hydrogen bonds. They are therefore relatively nonpolar, react slowly with diazo reagents, and require conjugation as a prerequisite to biliary excretion (4,11,12). Recognition of the existence and properties of these various bilirubin isomers has increased the understanding of the biologic properties of the naturally occurring 4*Z*, 15*Z*-IXα form and of the processes involved in its elimination by the liver. However, only the 4*Z*, 15*E* and 4*E*, 15*E* photoisomers, which are formed and readily excreted without conjugation during phototherapy for neonatal jaundice (41,42,43), are of clinical significance.

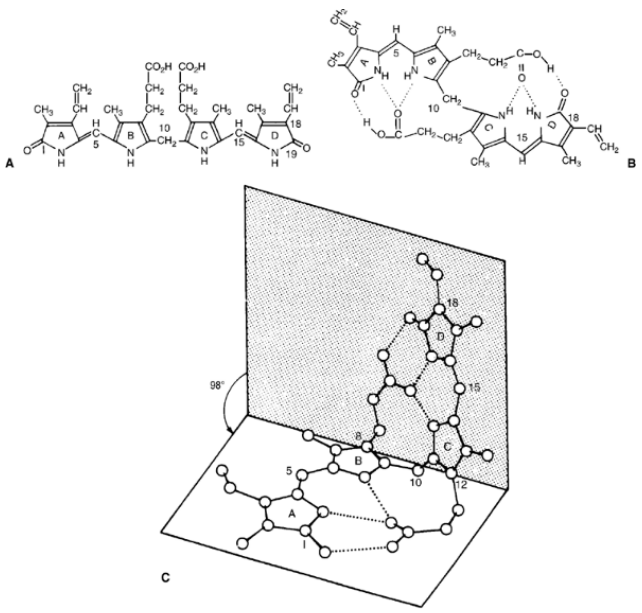
Bilirubin in Plasma

Although the bilirubin molecule contains two carboxyls and several other polar groups, internal hydrogen bonding involving these polar moieties constrains the molecule in a rigid, nonpolar, and, therefore, highly insoluble conformation. As an otherwise insoluble molecule, bilirubin formed in the periphery is transported to the liver tightly bound to albumin, at concentrations that far exceed its solubility in protein-free aqueous solutions (44,45,46). Adult human albumin has one high-affinity binding site for bilirubin and at least one class of lower-affinity sites. Experimental measurements of the affinity of bilirubin for albumin have varied considerably with the methods employed (44), but estimates of K_d for the high-affinity site have been in the micromolar range by several different approaches (44,45,47,48,49,50). The determination of these estimates has been based on the assumption that the affinity of bilirubin for albumin is constant and is independent of the albumin concentration. Under this assumption, until the bilirubin to albumin molar ratio in the circulation exceeds 1:1, virtually all the bilirubin present would be bound to the high-affinity site on albumin, and the unbound bilirubin concentration would remain extremely small. This small, unbound bilirubin concentration (51) is, nevertheless, considered to be an important driving force for hepatocellular bilirubin uptake (see following text). Under this model, if the 1:1 molar ratio of bilirubin to albumin is exceeded, the unbound bilirubin concentration increases rapidly with further increases in total bilirubin. In the neonatal period, increased levels of unbound bilirubin can cross the blood–brain barrier, leading to the serious neurologic consequences of kernicterus (52,53,54). Similar neurotoxicity may rarely occur in adolescents and

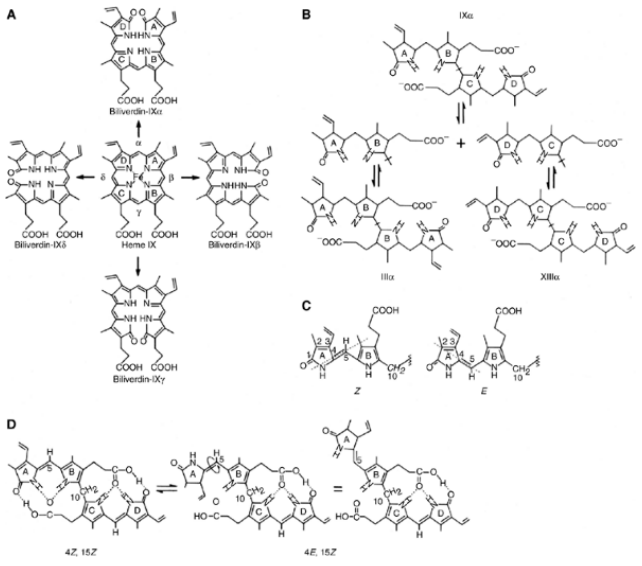
adults who develop sufficiently high concentrations of unconjugated bilirubin such that the critical 1:1 bilirubin to albumin molar ratio is exceeded (55). This is, in fact, the only clinically significant potential toxicity of hyperbilirubinemia. Because the normal albumin concentration is approximately 4 g/dL (600 μM), and a 1-mg/dL bilirubin concentration represents 17.1 μM, the critical 1:1 bilirubin to albumin molar ratio is usually exceeded in otherwise healthy adults only at bilirubin concentrations of 35 mg/dL or more. In catabolic states in which hypoalbuminemia exists, however, the 1:1 ratio may be exceeded at much lower bilirubin concentrations, for example, less than 17 mg/dL in the presence of an albumin concentration of 2 g/dL. Although models of bilirubin binding to albumin that assume a constant affinity independent

of the albumin concentration have been the basis for predictions of bilirubin concentrations at which the risk of kernicterus increases, several recent studies have challenged the basic assumptions of the model, reporting that the affinity of bilirubin for albumin actually varies inversely with the albumin concentration (51,56). This makes calculation of the critical unbound bilirubin concentration, and hence the risk of kernicterus, even more uncertain than earlier. However, the most rapid changes in affinity reportedly occur at quite low albumin concentrations, with only relatively minor further changes occurring as the albumin concentration is increased above 150 μM (56). Therefore, the impact of this new observation on bilirubin–albumin binding within the physiologic range of albumin concentrations, and hence on the risk of kernicterus, is yet to be definitely determined and may be very small. A variety of xenobiotics may displace or otherwise influence the binding of bilirubin to albumin. The resulting increase in the free bilirubin concentration may increase the risk of kernicterus in susceptible individuals (52,57,58,59).





▪ **Figure 8.3** Structure and conformation of bilirubin. **A:** Conventional "linear tetrapyrrole" structure of the naturally occurring isomer of bilirubin, designated bilirubin-IXα. The oxygen functions on the *A* and *D* rings are depicted as the lactam tautomers, and the bridge carbons at positions 5 and 15 are shown in the *Z* configuration. In this configuration the bridge carbons and their attached hydrogens project toward the substituted β positions on the adjacent pyrrole rings, just as in the protoporphyrin ring from which bilirubin is derived. **B:** Planar representation of the three-dimensional conformation of the bilirubin molecule, showing hydrogen bonding (…) between each of the –COOH side chains and the –C–O and –NH groups of the end (A and D) rings of the opposite half of the molecule. These hydrogen bonds, and weaker ones with -NH groups in the B and C rings, hold the molecule in a rigid three-dimensional conformation. **C:** Three-dimensional representation of bilirubin-IXα. The molecule takes the form of a ridge tile (i.e., a tile that fits along the top of a roof), with the ridge line defined by the carbons at positions 8, 10, and 12. Rings *A* and *B* lie in one plane, and *C* and *D* lie in another, with the interplanar angle being approximately 98 degrees. (Reprinted from reference Berk PD, Jones EA, Howe RB. Disorders of bilirubin metabolism. In: Bondy PK, Rosenberg LE, eds. *Metabolic control and disease*, 8th ed. Philadelphia: WB Saunders, 1979:1009–1088, with permission of the publisher.)



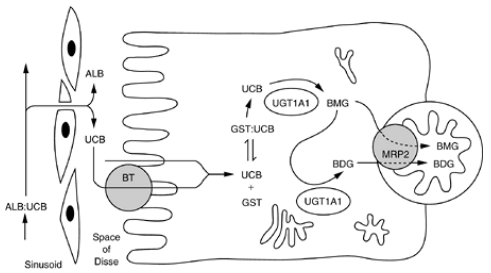
▪ **Figure 8.4** Bilirubin isomers. **A:** Formation of α, β, γ, and δ isomers of biliverdin by nonenzymatic cleavage of the protoporphyrin ring of heme at the α-, β-, γ-, and δ-bridge carbons, respectively. **B: Dipyrrolic scrambling**. This process involves the nonenzymatic dissociation of the bilirubin tetrapyrrole into dipyrrolic units, which may then reassemble at random into symmetric (bilirubin-IIIα and XIIIα) and nonsymmetric (bilirubin-IXα) tetrapyrroles. When this process occurs in a mixture of the C8 and C12 isomers of bilirubin-IXα monoglucuronide, the final products will include IIIα, IXα, and XIIIα isomers of both unconjugated bilirubin and its mono- and diglucuronides. **C: Nomenclature of the Z and E configurational isomers of bilirubin**. If a plane is erected perpendicular to the page along the 4, 5 double bond (illustrated by the *dashed lines*), the *B* ring may be together on the same side of the plane (*Z* [German: Zusammen]) or on opposite sides of the plane (*E* [Entgegen]) from the NH group in the *A* ring. In the *Z* configuration, the meso hydrogen at position 5 is trans to the A-ring lactam hydrogen, whereas in the *E* configuration it is cis. **D: E, Z isomerization at the 4,5 double bond**. In the 4*Z*, 15*Z* configuration, the molecule is rigidly hydrogen bonded. In the 4*E*, 15*Z* configuration, the *A*-ring nitrogen and oxygen groups are not spatially available to form hydrogen bonds with the C12 propionic acid side chain. Because of free rotation about the C5–6 bond, the two 4*E*, 15*Z* structures are equivalent. Analogous geometric isomerization may occur at the 15, 16 double bond. (Reproduced from Berk PD. Bilirubin metabolism and the hereditary hyperbilirubinemias. In: Berk JE, Haubrich MD, Kalser MH, et al. eds. *Bockus' gastroenterology*, Vol. 5. Philadelphia: WB Saunders, 1985:2732–2797, with permission of the publisher.)

Hepatic Disposition of Bilirubin

Because bilirubin is a potentially toxic waste product, its hepatic disposition is designed to eliminate it from the body through the biliary tract. Transfer of bilirubin from blood to bile involves four distinct but interrelated steps: Hepatocellular bilirubin uptake, binding to specific intracellular cytosolic proteins, conjugation with glucuronic acid, and canalicular excretion (Fig. 8.5) (5,7).

Bilirubin Uptake

The fenestrated endothelium that lines the hepatic sinusoids provides the bilirubin–albumin complex with ready access to the extrasinusoidal space of Disse, where it can come into direct contact with microvilli lining the sinusoidal surface of the hepatocytes (5,60,61). In this setting, bilirubin dissociates from albumin and is transported across the hepatocyte plasma membrane into the cell. Numerous in vivo studies of bilirubin uptake kinetics in animals; isolated, perfused livers; isolated hepatocytes; and plasma membrane vesicles have all indicated that bilirubin uptake is concentrative, saturable, and competitively inhibited by other organic anions such as sulfobromophthalein (BSP), implying a protein-mediated, facilitated uptake process (62,63,64,65,66,67,68,69,70,71). Subsequent reports, however, showed that BSP and bilirubin uptake could be dissociated under certain conditions, implying the existence of both shared and separate transport processes (66). Despite an intensive search, the putative bilirubin transporter has not yet been identified, and several candidate transporters (72), including such historical ones as BSP/bilirubin binding protein (BSP/BR-BP), organic anion binding protein (OABP), bilirubin translocase (BTL) (67), and the more recently reported human transporter SLC21A6 (68,69), have failed to withstand closer scrutiny. Recent studies have also identified a purely passive, nonsaturable bilirubin uptake process, but its relative magnitude compared with the saturable process remains to be determined (70). Although transporter-mediated hepatocellular bilirubin uptake is yet to be definitively identified, it appears that unconjugated bilirubin can be exported from the hepatocyte by the human multidrug resistance–associated protein 1 (MRP1) (71).



▪ **Figure 8.5** Hepatocellular transport of bilirubin. Efficient transfer of bilirubin from blood to bile is dependent on normal sinusoidal architecture, plasma membrane transport processes, and intracellular binding and conjugation. Albumin-bound bilirubin in sinusoidal blood passes through endothelial cell fenestrae to reach the hepatocyte surface, entering the cell by both facilitated and simple diffusional processes. Within the cell it is bound to glutathione-*S*-transferases and conjugated by bilirubin-uridine-5′-diphosphate-glucuronosyltransferase (UGT1A1) to monoglucuronides and diglucuronides, which are actively transported across the canalicular membrane into the bile. ALB, albumin; UCB, unconjugated bilirubin; BT, proposed bilirubin transporter; GST, glutathione-*S*-transferase; BMG, bilirubin monoglucuronide; BDG, bilirubin diglucuronide; MRP2, multidrug resistance–associated protein 2. (Reproduced from Berk PD, Wolkoff AW. Bilirubin metabolism and the hyperbilirubinemias. In: Braunwald E, Fauci AS, Kasper DL, et al. eds. *Harrison's principles of internal medicine*, 15 ed. New York: McGraw-Hill, 2001:1715–1720, with the permission of the publisher.)

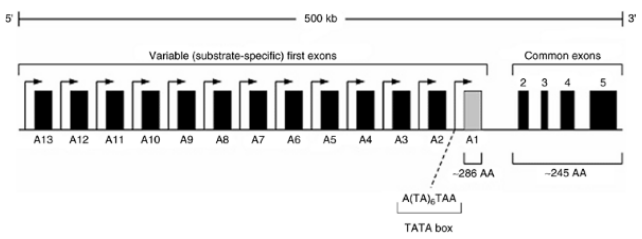
Intracellular Binding

Once within the cell, bilirubin partitions between the cytosol and the lipid bilayer of various intracellular membranes. As with bilirubin binding to albumin

in plasma, the cytosolic bilirubin fraction is kept in solution at concentrations that far exceed its aqueous solubility by binding as a nonsubstrate ligand to a number of proteins, of which the most abundant and best characterized are members of the glutathione-*S*-transferase (GST) superfamily (73,74). This family includes a large number of homodimeric and heterodimeric proteins, previously referred to as *ligandins* (75), that are principally responsible for bilirubin binding. Kinetic analyses suggest that binding to these proteins is not involved in the initial process of cellular bilirubin uptake but does increase net bilirubin sequestration by decreasing bilirubin efflux from the cytosol back into the space of Disse (76). The GSTs have been postulated to play a specific role in presenting bilirubin to the microsomes for subsequent conjugation (4,72).

Bilirubin Glucuronidation

The aqueous insolubility of bilirubin reflects the rigid, highly ordered, molecular structure conferred by internal hydrogen bonding that prevents solvent access to polar components of the molecule. Subsequent conjugation with glucuronic acid residues disrupts this internal hydrogen bonding, rendering the resulting monoglucuronide and diglucuronide conjugates highly soluble in aqueous solutions. The enzyme responsible for bilirubin glucuronidation is the uridine-5′-diphosphate glucuronosyltransferase isoform 1A1 (UGT1A1), which is encoded by the *UGT1* gene on chromosome 2 (77). This gene has a complex structure, and mutations within it are recognized as the cause of three different disorders characterized by unconjugated hyperbilirubinemia: Gilbert's syndrome and Crigler-Najjar syndrome types I and II. The *UGT1* gene (Fig. 8.6) (7) consists of 13 exons (designated A1 to A13) each of which encodes a distinct, substrate-specific binding site for one of the multiple protein isoforms produced by this single gene locus. Initiation of ribonucleic acid transcription at each of these 13 exons is controlled by a separate promoter element immediately upstream of its unique exon. Alternative splicing fuses one of these upstream exons with the four exons (exons 2 to 5) common to all UGT1 protein isoforms. Exon A1 and the four common exons code for the UGT1A1 protein that is responsible for glucuronidation of bilirubin (78) (Fig. 8.6).



▪ **Figure 8.6** Structural organization of the human *UGT1* gene complex. This large complex on chromosome 2 contains at least 13 substrate-specific first exons (A1, A2, etc.), each with its own promoter, that encode the *N*-terminal substrate-specific 286 amino acids (AAs) of the various *UGT1*-encoded isoforms, and common exons 2 to 5 that encode the 245 carboxyl-terminal amino acids common to all the isoforms. Messenger ribonucleic acids (mRNAs) for specific isoforms are assembled by splicing a particular first exon such as the bilirubin-specific exon A1 to exons 2 to 5. The resulting message encodes a complete enzyme, in this particular case bilirubin-uridine-5′-phosphate-glucuronosyltransferase (UGT1A1). Mutations in a first exon affect only a single isoform. Those in exons 2 to 5 affect all enzymes encoded by the UGT1 complex. (Reprinted from Berk PD, Wolkoff AW. Bilirubin metabolism and the hyperbilirubinemias. In: Braunwald E, Fauci AS, Kasper DL, et al. eds. *Harrison's principles of internal medicine*, 15 ed. New York: McGraw-Hill, 2001:1715–1720, with permission of the publisher.)

Canalicular Excretion of Bilirubin

Bilirubin glucuronides are transported across the apical plasma membrane into the canaliculus by an adenosine triphosphate (ATP)-dependent process mediated by a membrane protein initially called *canalicular multispecific organic anion transporter* (*cMOAT*), but now designated *MRP2* (Fig. 8.5) (79,80). MRP2 is a member of the MRP gene family, other members of which pump drug conjugates, as well as unmodified anticancer drugs, out of cells. In mouse models, effective MRP2 function requires the presence of at least one additional protein, radixin, which localizes to the canalicular membrane and directly binds the carboxyl-terminal cytoplasmic domain of MRP2 (81).

Fate of Bilirubin in the Gastrointestinal Tract

Conjugated bilirubin excreted in bile passes through the small intestine without significant absorption and reaches the colon intact (82,83,84,85). There, it is both deconjugated, presumably by bacterial β-glucuronidases (86), and degraded by other bacterial enzymes to a large series of urobilinogens and other products (87,88,89,90), the nature and relative proportions of which depend in part on the bacterial flora (84,91,92). Because of this variability, quantitation of fecal urobilinogen excretion does not provide an accurate measure of heme degradation and bilirubin formation (93) and has largely been abandoned as a clinical test for hemolysis or ineffective erythropoiesis.

Some urobilinogen is reabsorbed from the colon (94), resulting in small but measurable concentrations of urobilinogen in plasma (95). Most of this is re-excreted by the liver, but a small fraction is eliminated by the kidney. Increased urinary excretion of urobilinogen is a consequence of its increased plasma level. This in turn may reflect either increased bilirubin production, with a consequent increased formation and enterohepatic circulation of urobilinogen, or decreased hepatic clearance of urobilinogen. Hence, an elevated urine urobilinogen excretion does not distinguish between hemolysis and liver disease (96).

In the neonatal period, the presence of increased levels of intestinal β-glucuronidase (84,97) may result in the presence of appreciable amounts of unconjugated bilirubin within the distal small intestine and upper colon. Absorption from these sites can give rise to a significant enterohepatic circulation of unconjugated bilirubin (82,83,84), which has been implicated as a contributing factor to physiologic jaundice in the newborn and to the further increase in plasma bilirubin concentrations seen in neonates with intestinal obstruction, delayed passage of meconium, or fasting (86). In severe unconjugated hyperbilirubinemias such as those occurring in Crigler-Najjar syndrome type I or in the jaundiced Gunn rat (see following text), a similar enterohepatic circulation may result from unconjugated bilirubin being excreted both in bile (98,99) and directly across the intestinal lumen into the gut (98). Efforts to reduce unconjugated hyperbilirubinemia in such situations by interrupting the enterohepatic circulation of unconjugated bilirubin with the use of agents such as oral agar, charcoal, or cholestyramine have had at best limited and inconsistent success (84,86,100). Recent reports suggest that oral administration of calcium phosphate with or without the lipase inhibitor orlistat may be an efficient means to interrupt bilirubin enterohepatic cycling to reduce serum bilirubin levels (101,102).

Bilirubin in the Urine

Because of its very tight binding to albumin, the free fraction of unconjugated bilirubin in plasma is too small to permit efficient ultrafiltration at the glomerulus. Consequently, unconjugated bilirubin never appears in the urine no matter what its plasma concentration. By contrast, bilirubin conjugates are appreciably less tightly albumin bound. In the presence of cholestasis, whether secondary to hepatocellular injury or ductal obstruction, bilirubin conjugates formed in the hepatocyte are diverted back to the circulation, where their weaker albumin binding and larger free fraction permit excretion by the kidney, principally by glomerular filtration (103,104,105,106,107). A small degree of tubular reabsorption has been demonstrated, but tubular secretion apparently does not occur (107). The presence of bilirubin in the urine is an absolute indicator of conjugated hyperbilirubinemia.

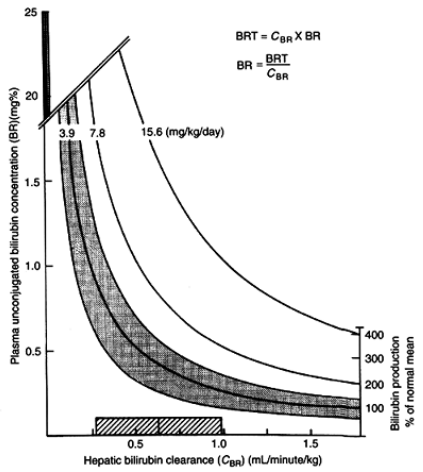
Clinical Physiology of Bilirubin

The quantity of unconjugated bilirubin in plasma, and hence the plasma unconjugated bilirubin concentration, reflects a balance between two processes: Bilirubin production and hepatic bilirubin clearance (Fig. 8.7) (5,24,27,108). This balance is indicated by the relationship:

BR [not asymptotically equal to] BRT/C_{BR},

where BR represents the plasma unconjugated bilirubin concentration, BRT is plasma bilirubin turnover (which closely approximates bilirubin production), and C_{BR} is the rate of clearance of unconjugated bilirubin from plasma by the liver. Measurement of C_{BR}, in units of milliliter per minute per kilogram, is a quantitative test of hepatic function that is, conceptually, analogous to creatinine clearance, a widely used measure of renal function. Both BRT and C_{BR} can be calculated from the area under the curve of an injected tracer dose of radiolabeled unconjugated bilirubin (24,27,108,109). Alternatively, the bilirubin production rate can be estimated by isotope dilution from the specific activity of fecal bile pigments after an intravenous injection of radiolabeled bilirubin (25,26,110), or from measurements of the excretion rate of CO (15,111,112). Although estimates of BRT and C_{BR} by any of these methods are not available as routine clinical measurements, an appreciation of the physiologic implications of these two variables is very useful in interpreting data that *are* readily available in clinical settings. Specifically, the equation indicates that BR is directly proportional to the rate of bilirubin turnover and inversely related

to hepatic bilirubin clearance in a manner analogous to the relationship between glomerular filtration rate and serum creatinine. Moreover, starting from any given baseline values of BRT and C_{BR}, a change in either BRT or C_{BR} will result in a corresponding fractional change in BR. The fractional change in BR will be directly proportional to any fractional change in BRT and inversely proportional to a fractional change in C_{BR} (Figs. 8.7 and 8.8) (27,108). As a result, for any given level of bilirubin production, equal *fractional* changes in hepatic bilirubin clearance can have dramatically different effects on plasma bilirubin concentrations, depending on the initial absolute value of C_{BR}. For example, when bilirubin turnover is normal (e.g., 4 mg/kg per day), reducing bilirubin clearance from a normal mean value of 0.70 mL/minute/kg to a lower mean value of 0.35 mL/minute/kg (a reduction of 50%) will approximately double the serum bilirubin concentration, increasing it by approximately 0.4 mg/dL (from 0.4 to 0.8 mg/dL). This increment may well go unnoticed clinically. By comparison, in a patient whose hepatic clearance is already reduced, a corresponding 50% reduction in bilirubin clearance, for example, from 0.1 to 0.05 mL/minute/kg, will again double the bilirubin concentration. In this instance, however, the interval increase, by approximately 2.7 mg/dL, will be sufficient to be clinically detectable. Similarly, doubling bilirubin production will double the plasma concentration of unconjugated bilirubin. The absolute magnitude of the increase, in milligram per deciliter, will depend on the value of C_{BR}.

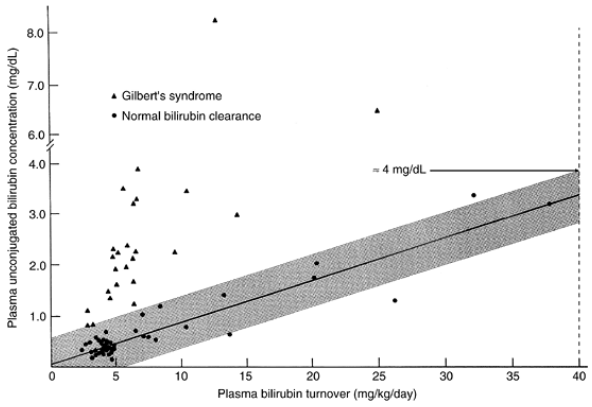


· **Figure 8.7** Relationship between plasma bilirubin turnover (BRT), hepatic bilirubin clearance (C_{BR}), and the plasma concentration of unconjugated bilirubin (BR). Stippled area represents the normal range for bilirubin turnover; bar on the horizontal axis is the normal range (mean ± 2 standard deviations) for C_{BR}. (Reprinted from Berk PD, Martin JF, Blaschke TF, et al. Unconjugated hyperbilirubinemia. Physiologic evaluation and experimental approaches to therapy. *Ann Intern Med* 1975;82:552–570, with permission of the publisher.)

Measurement of Plasma Bilirubin Concentration

Plasma bilirubin is typically measured in clinical laboratories by some modification of the diazo reaction first described by van den Bergh and Müller in 1916 (113) (reviewed in ref. 114). In this procedure, unconjugated

bilirubin in the sample reacts slowly with the diazo reagent (e.g., diazotized sulfanilic acid) because the central bridge carbon, which is the site of the attack by the reagent, is rendered sterically inaccessible by internal hydrogen bonding within the molecule (Fig. 8.3). In the presence of ethanol, caffeine, or other "accelerators" that disrupt the internal hydrogen bonding, the central bridge carbon becomes more readily accessible to nucleophilic attack and the unconjugated bilirubin molecule reacts more rapidly and completely. Similarly, rapid diazo reactivity is displayed by conjugated bilirubin, in which esterification of propionic acid side chains with glucuronic acid prevents hydrogen bond formation and exposes the central bridge carbon. Accordingly, the "prompt" or *direct-reacting bilirubin* in serum or plasma, considered a measure of the amount of conjugated bilirubin present, is determined a short time (30 to 60 seconds) after the addition of the diazo reagent to the sample in the absence of an accelerator. The *total bilirubin* concentration, a measure of both unconjugated and conjugated bilirubin, is typically measured at some more prolonged interval (e.g., 30 to 60 minutes) after addition of an accelerator substance. The *indirect-reacting* bilirubin, calculated as the difference between the total and the direct-reacting bilirubin, is widely used as a proxy for the amount of unconjugated bilirubin in the sample (114).



· **Figure 8.8** Relationship between plasma bilirubin turnover and the plasma concentration of unconjugated bilirubin. Normal plasma bilirubin turnover is up to 5 mg/kg body weight per day. Higher values indicate increased bilirubin production, usually from hemolysis. When hepatic bilirubin clearance is within the normal range, the plasma unconjugated bilirubin concentration increases linearly with increases in bilirubin turnover, as indicated by the regression line (the stippled area represents ± 2 standard errors of the estimate about the regression line). Extrapolation of the regression line to the maximal rate of steady state bilirubin production indicates the highest level of unconjugated hyperbilirubinemia that can result from sustained hemolysis in an individual with normal hepatic bilirubin clearance. Because the bone marrow can increase erythrocyte production only by about eightfold in response to hemolysis, the maximum sustainable rate of bilirubin turnover is approximately 40 mg/kg per day, and the corresponding value of plasma unconjugated bilirubin is 4 mg/dL. (Reproduced from Berk PD, Martin JF, Blaschke TF, et al. Unconjugated hyperbilirubinemia. Physiologic evaluation and experimental approaches to therapy. *Ann Intern Med* 1975;82:552–570, with permission of the publisher.)

Bilirubin can also be estimated in biologic fluids by direct spectrophotometry because of its intense absorption band at approximately 450 nm (12,115). The method is rapid, requires very small samples, and is therefore often used in neonatal nurseries or in amniotic fluid analyses (116) in which sample availability is limited. The method is nonspecific because turbidity and other yellow–orange materials such as carotenoids interfere. Various devices designed to measure bilirubin levels transcutaneously without blood sampling, by reflectance and/or spectrophotometry, are widely used in neonatal nurseries (see for example ref. 117). Accurate alternative methods also exist for the quantification of individual bilirubin species (118,119), including bilirubin conjugates covalently bound to plasma proteins (i.e., δ-bilirubin, see following text), not only in plasma but also in bile, urine, and other biologic

fluids (120,121,122,123,124,125). Because they are rarely employed outside research laboratories, a description of these technologies is beyond

the scope of this review.

Normal Ranges

Using conventional, diazo-based analytic procedures, the upper limit of normal for *total plasma bilirubin* has been reported to be anywhere between 1.0 and 1.5 mg/dL (17 and 26 μM), and that for the indirect-reacting fraction between 0.8 and 1.2 mg/dL (14 and 21 μM) (2,96,114,126,127). Differences reported reflect both variations in analytic methods and whether the 95% or 99% confidence limit was used to define the normal range. They also reflect the fact that bilirubin concentrations in the healthy population exhibit a log-normal (skewed to the right) rather than a Gaussian (bell-shaped) distribution, which must be accounted for in establishing appropriate normal limits (126).

Many studies have set the upper limit of normal for the *indirect-reacting fraction* at 1.0 mg/dL (17 μM), which is in close agreement with the limits predicted on theoretic grounds from knowledge of the distribution of plasma bilirubin turnover and hepatic bilirubin clearance rates in a healthy population (126). When the total plasma bilirubin concentration is normal, the normal direct-reacting fraction (a proxy for conjugated bilirubin) is traditionally reported to be less than 0.1 mg/dL, or at most less than 0.2 mg/dL (114). Because unconjugated bilirubin does react, although slowly, with diazo reagents even in the absence of an accelerator, even this small direct-reacting fraction *overestimates* the miniscule amounts of conjugated bilirubin actually present (125). Consequently, the calculated indirect-reacting bilirubin *underestimate*s the amount of unconjugated bilirubin present. The proportional magnitude of these errors is greatest at total bilirubin concentrations within or near the normal range. Nevertheless, at virtually any total bilirubin level, if the direct-reacting fraction is less than 15% of the total, the bilirubin in the sample can be considered essentially as being completely unconjugated. Unfortunately, the errors involved appear to be greater with autoanalyzer methods currently in widespread use than they were with manual methods used in the past. "Normal" values for direct-reacting bilirubin have been creeping upward over the years even as more precise chromatographic methods demonstrate that the actual amounts of conjugated bilirubin in normal serum or plasma are vanishingly small. An informal survey several years ago suggested that few large laboratories in the New York metropolitan area set an upper limit of normal for direct-reacting bilirubin as low as 0.2 mg/dL. The most commonly reported upper limit was 0.3 mg/dL, with limits of 0.4 to 0.8 mg/dL being reported not infrequently. Such latitude can lead to considerable error in interpreting the direct- and indirect-reacting fractions as conjugated and unconjugated bilirubin, respectively. Because the presence of even modest amounts of true conjugated bilirubin in serum should alert the clinician to the possibility of significant hepatobiliary pathology, this distinction is of more than academic interest (128). Because conventional bilirubin glucuronide conjugates are water soluble and bind relatively loosely to albumin, they are readily filtered at the glomerulus and excreted in the urine. Accordingly, uncertainty about the clinical significance of a mildly elevated level of direct-reacting bilirubin can often be clarified by a simple dipstick test for bilirubin in the urine. Even minimal degrees of conjugated hyperbilirubinemia are associated with bilirubinuria. A negative dipstick test in the presence of a modestly elevated direct-reacting fraction suggests the presence of either δ-bilirubin (see following text) or an artifact of the diazotization procedure.

With prolonged conjugated hyperbilirubinemia, some of the conjugated bilirubin in plasma binds *covalently* to albumin and produces what is designated the δ-bilirubin fraction (121,122,123,124). Although δ-bilirubin gives a direct diazo reaction, it is not filterable by the glomerulus and does not appear in the urine; it disappears slowly from the plasma with the 14- to 21-day half-life of the albumin to which it is bound. δ-Bilirubin accounts for the sometimes slow rate with which conjugated (direct) hyperbilirubinemia resolves as hepatitis improves or biliary obstruction is relieved. Although δ-bilirubin is not easily measured, its presence can be inferred when an elevated level of direct-reacting bilirubin persists after bilirubinuria resolves.

Hyperbilirubinemia and Jaundice

Hyperbilirubinemia is conveniently classified as *unconjugated (indirect-reacting), or conjugated (direct-reacting) hyperbilirubinemia*. In practice, pure conjugated hyperbilirubinemia is uncommon; in most cases an elevated plasma conjugated bilirubin level is accompanied by an elevation of the unconjugated bilirubin level, resulting in mixed hyperbilirubinemia. In this setting, because the plasma level of conjugated bilirubin reflects renal as well as hepatic clearance of bilirubin conjugates, the ratio of conjugated to total bilirubin is usually not helpful diagnostically. *Another useful characteristic is whether hyperbilirubinemia is the only abnormality of hepatic function or whether other hepatic biochemical tests such as the activities of serum aminotransferases* (*alanine aminotransferase [ALT], aspartate aminotransferase [AST]*), *alkaline phosphatase, or γ-glutamyltransferase are also abnormal*. Absence of abnormalities of other hepatic biochemical tests is one of the features that helps distinguish the *familial*

hyperbilirubinemias from most acquired cases of hyperbilirubinemia. However, certain forms of acquired hyperbilirubinemia such as acquired hemolytic disease and inactive cirrhosis may also occur in the absence of other biochemical abnormalities. The major causes of familial hyperbilirubinemia are listed in Table 8.1.

Table 8.1. The Familial Hyperbilirubinemias

I. Unconjugated hyperbilirubinemias
  A. From increased bilirubin production
    1. Hemolytic anemias
      a. Hemoglobinopathies
      b. Thalassemia syndromes
      c. Enzyme defects
      d. Membrane defects, etc.
    2. Shunt hyperbilirubinemias
      a. Congenital dyserythropoietic jaundice syndromes
      b. Miscellaneous
  B. From defective hepatic bilirubin clearance
    1. Gilbert's syndrome
    2. Crigler-Najjar syndrome
      a. Type I—phenobarbital resistant
      b. Type II—phenobarbital responsive
II. Conjugated hyperbilirubinemias
  A. Dubin-Johnson syndrome
  B. Rotor's syndrome

Causes and Consequences of Hyperbilirubinemia

Unconjugated hyperbilirubinemia

Unconjugated hyperbilirubinemia is the result of any process that increases bilirubin production, decreases bilirubin clearance, or results in both processes acting in concert (3,27,108,109). The reference range for the plasma unconjugated (i.e., indirect-reacting) bilirubin concentration is generally reported to be 0.3 to 1.0 mg/dL, although some laboratories set the upper limit at 1.2 mg/dL and occasionally as high as 1.5 mg/dL. Values above the reference range represent unconjugated hyperbilirubinemia. Although scleral icterus may become detectable when the bilirubin concentration exceeds 2.5 to 3.0 mg/dL, many cases of unconjugated hyperbilirubinemia are subclinical and detectable only by measurement of the plasma bilirubin concentration.

Increased bilirubin production

Hemolysis and increased ineffective erythropoiesis are two of the most common processes responsible for increased bilirubin production. A large number of distinct hereditary hemolytic disorders have been described (129) and result from inherited hemoglobinopathies, enzyme deficiencies,

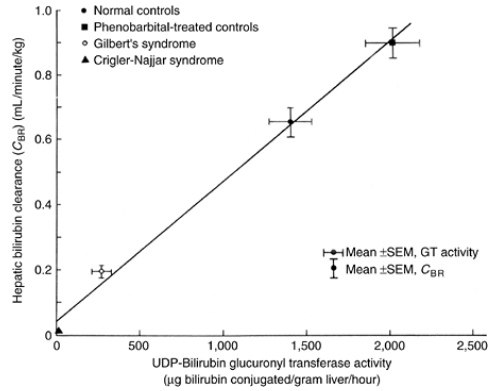
or abnormalities in red blood cell membrane structure. There are also many acquired hemolytic conditions, ranging from pure, specific autoimmune hemolytic anemias to the shortened red cell life spans that accompany many chronic diseases. Excessive ineffective erythropoiesis may occur on a congenital basis, as in any subtype of congenital dyserythropoietic anemia (3,5,28), or on an acquired basis, in disorders such as erythropoietic porphyria, pernicious anemia, and lead poisoning (2,29,129). Transfusion of old bank blood and massive hematomas or pulmonary infarcts can produce unconjugated hyperbilirubinemia by temporarily increasing bilirubin production. Excessive *hepatic* bilirubin production, although seemingly a potential cause of increased plasma bilirubin turnover, has not been convincingly documented as a cause for clinically evident hyperbilirubinemia. Although hepatic heme turnover has been reported to contribute as much as one fourth of the total bilirubin production on the basis of the labeling of plasma bilirubin after administration of radiolabeled heme precursors (see for example refs 130–132), approximately half of hepatic-derived bilirubin is excreted into the bile without transit through the plasma (2,5,24,25,27,110,131,133). This means that hepatic hemes contribute no more than approximately 12% of the plasma bilirubin turnover. Studies documenting a significant discrepancy between the increase in bilirubin labeling from ^{3}H- or ^{14}C-labeled heme precursors and total bilirubin production suggest that the actual percentage of plasma bilirubin turnover derived from hepatic hemes is appreciably lower (134,135). On this basis, hepatic heme turnover would have to increase manyfold to result in a clinically recognizable increase in the plasma unconjugated bilirubin concentration.

Unconjugated hyperbilirubinemia solely due to increased bilirubin production rarely exceeds 4 mg/dL, even in the face of brisk hemolysis (Fig. 8.8) and is generally well tolerated. However, subjects are at an increased risk for the development of pigmented gallstones.

Decreased bilirubin clearance

As noted in preceding text, four distinct processes are involved in hepatocellular bilirubin disposition, and each, if defective, can result in a decrease in hepatic bilirubin clearance. Although the precise mechanism(s) by which bilirubin is taken up by hepatocytes remains unclear, several drugs (e.g., rifampin, flavispidic acid, novobiocin, and various cholecystographic contrast agents) are reported to competitively inhibit the bilirubin uptake process (see ref. 5). The resulting unconjugated hyperbilirubinemia resolves with the cessation of the medication. Reduced hepatic bilirubin uptake (and net clearance) can also result from portosystemic shunting by which blood bypasses the hepatocytes. Although this is most commonly thought

of as occurring through venous channels such as varices, it may also result from capillarization of the hepatic sinusoids—that is, the loss of sinusoidal endothelial fenestrae and increased perisinusoidal matrix deposition—that occurs in cirrhosis. It is also a consequence of an absolute reduction in hepatic blood flow or of abnormalities in the net extraction of bilirubin from the circulation by hepatocytes.



· Figure 8.9 Relationship between the mean values for hepatic bilirubin clearance (C_{BR}) and bilirubin uridine-5′-diphosphate-glucuronosyltransferase (UDPGT) activity in patients with Crigler-Najjar syndrome type I, Gilbert's syndrome, and healthy controls. For the control group, data are presented for both untreated and phenobarbital-treated subjects. The line represents a least-squares fit to the mean values for the four groups. Subsequent data in Crigler-Najjar type II fell on the regression line. Data such as these suggested that Gilbert's syndrome and the two Crigler-Najjar syndromes might all reflect mutations with quantitatively differing effects on a single gene, designated at the time as bilirubin-UDP-glucuronyltransferase. (Modified from Blaschke TF, Berk PD, Rodkey FL, et al. Drugs and the liver. I. Effects of glutethimide and phenobarbital on hepatic bilirubin clearance, plasma bilirubin turnover and carbon monoxide production in man. *Biochem Pharmacol* 1974;23:2795–2806.)

Abnormalities in the binding of bilirubin to its cytosolic binding proteins are at least a hypothetical basis for decreased bilirubin clearance. However, defective glucuronidation is a more common mechanism that results in reduced bilirubin clearance and consequent unconjugated hyperbilirubinemia. Delayed expression of UGT1A1 in neonates is primarily responsible for the physiologic jaundice of otherwise healthy newborns. Peak serum bilirubin levels in this setting are typically less than 5 to 10 mg/dL between days 2 and 5 and decline to normal within 2 weeks. Higher neonatal bilirubin levels that predispose to kernicterus occur in the face of profound prematurity, Gilbert's syndrome (see following text), hemolysis, or both (see for example ref. 136). Three familial disorders of bilirubin conjugation are well recognized: Crigler-Najjar syndrome types I and II and Gilbert's syndrome. These are described in greater detail in following text. Although considered until recently as distinct disorders, these conditions are all now known to result from mutations of different functional severity in the bilirubin-conjugating enzyme UGT1A1. Acquired deficiency of UGT1A1 and consequent unconjugated hyperbilirubinemia also occurs with administration of certain human immunodeficiency virus protease inhibitors such as indinavir and atazanavir (reviewed in ref. 137,138).

Conjugated hyperbilirubinemia

Conjugated hyperbilirubinemias typically reflect abnormalities in hepatocellular excretion of conjugated bilirubin or in biliary tract obstruction. Dubin-Johnson and Rotor's syndromes are uncommon, heritable disorders of conjugated bilirubin excretion. In Dubin-Johnson syndrome, mutations in MRP2 result in deficient canalicular transport of bilirubin conjugates (80,139,140). The molecular defect in Rotor's syndrome remains unknown but produces a phenotype similar in many respects to that of Dubin-Johnson. In both disorders, general hepatocellular function is preserved and liver chemistries other than the bilirubin

concentration are typically normal. Bilirubin concentrations in Dubin-Johnson and Rotor's syndromes are most often between 2 and 5 mg/dL, although values as high as 5 mg/dL for prolonged periods have been described. Extensive clinical experience suggests that conjugated hyperbilirubinemia produces no significant adverse consequences, even if prolonged for months at levels of up to 35 to 40 mg/dL.

Far more common is the defective bilirubin excretion that occurs with a broad spectrum of hepatobiliary diseases. In these conditions, elevations in bilirubin concentration typically occur in association with abnormalities of other hepatic biochemical tests, including elevations in AST and ALT levels, alkaline phosphatase level, and, if severe, reduction in serum albumin level and prolongation of clotting times. This broad category of diseases includes hepatocellular and cholestatic liver diseases, benign postoperative jaundice, mechanical intrahepatic or extrahepatic bile duct obstruction, and a rare group of disorders classified under the rubric of familial intrahepatic cholestasis (141).

Familial Hyperbilirubinemias

The Familial Unconjugated Hyperbilirubinemias

The spectrum of familial unconjugated hyperbilirubinemias is indicated in Table 8.2. This review limits itself to those entities associated with a decrease in hepatic bilirubin clearance: Crigler-Najjar syndrome types I and II and Gilbert's syndrome. Important characteristics of these three entities are summarized in Table 8.2. These were once considered distinct genetic and pathophysiologic entities, with Gilbert's syndrome reportedly an autosomal dominant disorder and Crigler-Najjar type I an autosomal recessive disorder (see for example refs 2,4,5). However, physiologic observations (Fig. 8.9) (142) suggested that the three entities might reflect mutations with quantitatively different impact on the functioning of a single gene. Subsequent molecular findings in the specific syndromes and the observation that one *normal* UGT1A1 allele is sufficient to maintain a normal plasma bilirubin concentrations (77,78) have established that in almost all instances the hereditary unconjugated hyperbilirubinemias are related autosomal recessive disorders.

Crigler-Najjar syndrome type I

Crigler-Najjar syndrome type I is a rare, recessive disorder characterized by profound unconjugated hyperbilirubinemia, with bilirubin concentrations of 20 to 45 mg/dL as a result of mutations in UGT1A1 that result in the near total loss of UGT1A1 enzyme activity (77,78,100,143,144,145,146,147,148,149,150,151). Mutations most often occur in exons 2 to 5 of the *UGT1* gene, affecting the glucuronidation of a wide spectrum of substrates in addition to bilirubin (type Ia). Less often, the mutation occurs in exon 1, and the loss of glucuronidation capacity is largely limited to bilirubin conjugation (type Ib). Crigler-Najjar syndrome type I first appears in the neonatal period, and historically, most patients have succumbed from kernicterus in infancy and early childhood. Patients with Crigler-Najjar syndrome type I do not respond to phenobarbital with a reduction in plasma bilirubin concentrations (2,4,6,8,9,10,11,100,146). Although survival has been extended with the advent of phototherapy, those who survive beyond early childhood remain at substantial risk for late-onset bilirubin encephalopathy, which often sets in after even mild febrile illnesses (see for example refs 100,152). Although isolated hepatocyte transplantation has been used experimentally in a limited number of cases of Crigler-Najjar syndrome type I (153,154), early liver transplantation remains the best hope to prevent brain injury and death (155,156,157,158).

Much of the basis for elucidation of the pathobiology of Crigler-Najjar syndrome type I has arisen from studies that have been performed in the Gunn rat. This mutant Wistar strain of rats was initially described by Gunn in 1938 as having chronic nonhemolytic unconjugated hyperbilirubinemia (159). As in patients with Crigler-Najjar syndrome type I, jaundice in these animals is inherited as an autosomal recessive trait. Heterozygotes are anicteric, and liver histology in the affected rats is normal. Bilirubin glucuronyl transferase activity is undetectable in the livers of these rats (160).

Crigler-Najjar syndrome type II

In contrast to Crigler-Najjar type I, in Crigler-Najjar type II UGT1A1 activity is maintained, although at a minimal level (<10% of normal), and serum bilirubin concentrations typically vary between 6 and 25 mg/dL (146,161,162). Induction of UGT1A1 levels with exposure to phenobarbital can further reduce bilirubin levels by more than 25% (146,163,164,165,166). Depending on the severity of the molecular defect, either basal or phenobarbital-stimulated enzyme activity is sufficient in most cases to prevent the development of kernicterus. Clinically relevant neurologic sequelae can, however, be precipitated in the setting of intercurrent illnesses, fasting, or any other factor that temporarily raises the serum bilirubin concentration significantly above baseline (see for example ref. 55), especially if the resulting bilirubin to albumin molar ratio exceeds 1. Overall, available data in most patients with type II syndrome indicates that in adolescents and adults with normal serum albumin levels, the prolonged exposure to unconjugated bilirubin levels below 16 mg/dL

does not result in neurologic injury. Indeed, most patients in the original reports describing the syndrome were healthy adults, many with college-level education (146,161). To date, a total of 77 different mutations in the *UGT1* gene have been identified in association with either type I or type II Crigler-Najjar syndrome (167). Chain-terminating mutations have been more commonly associated with a Crigler-Najjar type I phenotype, whereas missense mutations have been more frequently observed among less severely affected patients with Crigler-Najjar syndrome type II (167).

Table 8.2. Principal Features of the Familial Unconjugated Hyperbilirubinemias

	Crigler-Najjar Syndrome		
Feature	**Type I**	**Type II**	**Gilbert's syndrome**
Incidence	Very rare	Uncommon	Up to 12% of population
Total serum bilirubin (mg/dL)	18–45 (usually >20), unconjugated	6–25 (usually ≤20), unconjugated	Typically ≤4 in the absence of fasting or hemolysis; mostly unconjugated
Defect(s) in bilirubin metabolism	Bilirubin UGT1A1 activity markedly reduced: Trace to absent	Bilirubin UGT1A1 activity reduced: ≤10% of normal	Bilirubin UGT1A1 activity typically reduced to 10%–33% of normal; reduced hepatic bilirubin uptake in some cases; mild hemolysis in up to 50% of patients
Routine liver tests	Normal	Normal	Normal
Serum bile acids	Normal	Normal	Normal
Plasma sulfobromophthalein removal (percentage retention of 5 mg/kg dose at 45 min)	Normal	Normal	Usually normal (<5%); mildly increased 45-min retention (<15%) in some patients
Oral cholecystography	Normal	Normal	Normal
Pharmacologic responses/special features	No response to phenobarbital	Phenobarbital reduces bilirubin by ≤75% but not to normal	Phenobarbital reduces bilirubin, often to normal
Major clinical features	Kernicterus in infancy if untreated; may occur later despite therapy	Rarely late-onset kernicterus with fasting	None

Hepatic morphology/histology	Normal	Normal	Normal; occasionally increased lipofuscin pigment
Bile bilirubin fractions[a]	>90% unconjugated	Largest fraction (mean 57%) monoconjugates	Mainly diconjugates but monoconjugates are increased (mean 23%)
Inheritance (all autosomal)	Recessive	Recessive	Promoter mutation is recessive; missense mutation often dominant
Diagnosis	Clinical and laboratory findings; lack of response to phenobarbital	Clinical and laboratory findings; response to phenobarbital	Clinical and laboratory findings; promoter genotyping may be helpful; liver biopsy rarely necessary
Treatment	Phototherapy or tin protoporphyrin as short-term therapy; liver transplantation definitive	Consider phenobarbital if baseline bilirubin ≥8 mg/dL	None necessary

[a]Bilirubin in normal bile: <5% unconjugated bilirubin, with an average of 7% bilirubin monoconjugates and 90% bilirubin diconjugates.
UGT1A1, uridine diphosphate glucuronosyltransferase isoform 1A1.

Gilbert's syndrome

Gilbert's syndrome resides at the other end of the spectrum of disorders of bilirubin conjugation. Originally described in 1901 (168), this condition occurs with a phenotypic prevalence of approximately 8% in the general population and is characterized by a mild, unconjugated hyperbilirubinemia, with bilirubin levels that rarely exceed 4 mg/dL; an otherwise normal liver function; and a hepatic histology that is also normal other than a modest increase in lipofuscin

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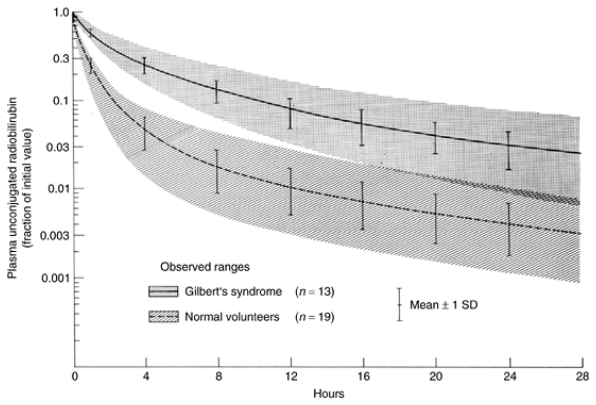
pigment in some cases. Other than mild icterus in some patients, physical examination is also unremarkable. Bilirubin clearance is reduced to approximately one third of normal (Fig. 8.10) (169), and some patients also exhibit abnormalities in the plasma clearance patterns of other organic anions such as BSP or indocyanine green (ICG) (64,170), for which the mechanism remains uncertain. By contrast, fasting serum bile acid levels are normal (171). The most commonly recognized molecular defect is the addition of an extra dinucleotide sequence, TA, to the transcription initiation sequence (TATAA box) of the promoter for the A1 exon (172). Therefore, compared with the normal $A(TA)_6TAA$ sequence, an $A(TA)_7TAA$ mutation is commonly found in patients with Gilbert's syndrome. Much less common $A(TA)_5TAA$ or $A(TA)_8TAA$ mutations are also associated with Gilbert's syndrome (173). In individuals homozygous for one of these variants, enzyme activity is decreased to 10% to 35% of normal because of decreased synthesis of a functionally normal enzyme. Homozygosity for the promoter mutation appears to be necessary, but apparently not always sufficient for clinical expression of Gilbert's syndrome because population studies suggest that only about half of $A(TA)_7TAA$ homozygotes have hyperbilirubinemia (174). It has been suggested that additional variables, such as mild hemolysis (reported in up to 50% of patients with Gilbert's syndrome) or a separate defect in bilirubin uptake (7,169,172,173,175), might be among the factors enhancing phenotypic expression.



▪ **Figure 8.10** Observed ranges of plasma radiobilirubin disappearance curves in patients with Gilbert's syndrome and in healthy young adult volunteers. The average curve for each group was calculated by the computer from the mean values, for the group, of the intercompartmental rate constants (λs) of a compartmental model of bilirubin metabolism. The groups do not overlap for the first 16 hours after injection. Estimates of bilirubin clearance (C_{BR}), calculated from the areas under the curves, were reduced to one third of normal in the patients with Gilbert's syndrome. (Reprinted from Berk PD, Martin JF, Blaschke TF, et al. Unconjugated hyperbilirubinemia. Physiologic evaluation and experimental approaches to therapy. *Ann Intern Med* 1975;82:552–570, with permission of the publisher.)

Additional molecular mechanisms result in a phenotype identical to that associated with homozygosity for the $A(TA)_7TAA$ promoter variant (Table 8.3) (172,173). Consistent with the previously noted observation that one *normal* UGT1A1 allele is sufficient to maintain a normal plasma bilirubin concentration, individuals who are heterozygous for one UGT1A1 allele encoding an enzyme with reduced to absent bilirubin-conjugating activity will nevertheless have normal bilirubin concentrations if the second UGT1A1 allele is normal. However, if that second allele has the $A(TA)_7TAA$ promoter variant, the patient will have mild unconjugated hyperbilirubinemia with a

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Gilbert's syndrome phenotype. Additionally, a number of instances have been documented in which mutations in the coding regions of the UGT1A1 gene encode proteins with only mildly reduced enzymatic activity. Such missense mutations, which result in a Gilbert's syndrome phenotype, have thus far been reported only from Japan (176). Inheritance has been either recessive or dominant negative (172,173). If a phenotypic reduction in bilirubin clearance is taken as the operational definition of Gilbert's syndrome, recognition of these alternative genotypes is important for the appropriate interpretation of mild, nonhemolytic, unconjugated hyperbilirubinemia. Although homozygosity for the $A(TA)_7TAA$ promoter variant supports a diagnosis of Gilbert's syndrome, absence of homozygosity for this variant does not exclude Gilbert's syndrome in this setting.

Table 8.3. Alternative Molecular Bases for the Gilbert's Syndrome Phenotype

- $A(TA)_7TAA$ homozygote
- $A(TA)_7TAA$/structural mutation compound heterozygote
- Structural mutation, for example, G71R (homozygous recessive or dominant negative)

The diagnosis of Gilbert's syndrome is most often made clinically on the basis of a mild, unconjugated hyperbilirubinemia in the absence of other causes. Although a definitive diagnosis can be established by assays of UGT1A1 enzymatic activity or by the identification of the homozygous $A(TA)_7TAA$ promoter mutation, elaborate studies, such as liver biopsies, are rarely necessary (177). Provocation tests such as a 48-hour fast or the intravenous administration of nicotinic acid augment the bilirubinemia in patients with Gilbert's syndrome and in healthy controls by a similar proportion and are of limited value in establishing a diagnosis of Gilbert's syndrome (reviewed in ref. 5). Bilirubin levels in simple Gilbert's syndrome in adults are never sufficiently elevated to pose a risk of neurologic damage. However, neonates with *both* Gilbert's syndrome *and* some form of hemolysis are at increased risk of transiently developing dangerous degrees of hyperbilirubinemia (178,179,180,181).

Some patients with Gilbert's syndrome exhibit abnormalities in the hepatic handling of a variety of xenobiotics metabolized by glucuronidation, including menthol, estradiol benzoate, lamotrigine, tolbutamide, rifamycin SV, acetaminophen (5,182), and HIV protease inhibitors (137). The HIV protease inhibitors indinavir and atazanavir produce hyperbilirubinemia by specifically inhibiting UGT1A1 (138). The degree of hyperbilirubinemia is greater with preexisting Gilbert's syndrome. Abnormalities in the glucuronidation of other substrates may be attributable to polymorphisms of other UGT isoforms (182). No significant toxicity has been ascribed to any of these pharmacokinetic abnormalities. Virtually the sole risk from Gilbert's syndrome in adults is associated with exposure to the antitumor agent irinotecan (CPT-11), the active metabolite of which is glucuronidated by UGT1A1. Administration of CPT-11 to patients with Gilbert's syndrome has resulted in severe toxicities, including intractable diarrhea and myelosuppression.

The Familial Conjugated Hyperbilirubinemias

Two inherited disorders characterized by conjugated hyperbilirubinemia without cholestasis, the Dubin-Johnson (183) and Rotor's syndrome (184), have been described. Although a third disorder that had been termed *hepatic storage disease* (185) was also included in this group, more recent evidence indicates that these patients have Rotor's syndrome. These disorders are relatively rare. They are clinically benign, but the establishment of a precise diagnosis is important to differentiate them from other more serious disorders and to save patients from unnecessary anxiety or surgical intervention. There are several additional familial disorders characterized by conjugated hyperbilirubinemia in association with cholestasis. These include benign recurrent intrahepatic cholestasis (BRIC) (186,187), and the progressive familial intrahepatic cholestasis (PFIC) disorders (188).

Dubin-Johnson syndrome

Clinical features

This disorder, independently described in 1954 by Dubin and Johnson (183) and by Sprinz and Nelson (189), is characterized by mild, predominantly conjugated hyperbilirubinemia (Table 8.4). Aside from jaundice, physical examination is normal in most cases, but an occasional patient may be found to have hepatosplenomegaly (190,191). Mild constitutional symptoms similar to those observed in Gilbert's syndrome (i.e., vague abdominal pain, fatigue, and weakness) are common (190,191). However, as in Gilbert's syndrome, these symptoms may be related to the anxiety associated with prolonged diagnostic testing. For the most part, newly detected cases are asymptomatic. Hyperbilirubinemia and clinical icterus are typically increased by intercurrent illness, by the administration of drugs that decrease hepatic excretion of organic anions (notably oral contraceptives), and by pregnancy (192). The condition is rarely observed before the onset of puberty, although occasional cases have been reported in infants (193,194,195). Subclinical cases often become manifest during pregnancy or in association with the initiation of oral contraceptive therapy (192). In contrast to syndromes associated with true cholestasis, pruritus is not seen in Dubin-Johnson syndrome, and serum bile acid levels are characteristically normal (192,196) as are other routine test results of liver function (e.g., serum alkaline phosphatase) (183,191). Useful animal models of the Dubin-Johnson syndrome have been described in mutant Corriedale sheep (197,198,199,200), golden lion tamarin monkeys (201), and in transport-deficient/Groningen yellow (Tr^-/GY) mutant rat strains (80,202,203,204,205,206) and Eisai hyperbilirubinemic rat

(EHBR) (207,208,209) mutant strains. Because of the insights they have provided into the probable pathophysiology of the Dubin-Johnson syndrome, the rat mutants are discussed in detail in following text.

Table 8.4. Phenotypic Features of Dubin-Johnson and Rotor's Syndromes

Feature	Dubin-Johnson syndrome	Rotor's syndrome
First described	1954	1948
Serum bilirubin (mg/dL)	Usually 2–5 mg/dL, predominantly (~60%) direct reacting; less often ≤25 mg/dL total	Usually 2–5 mg/dL, predominantly (~60%) direct reacting; less often ≤25 mg/dL total
Other liver function tests	Normal	Normal
Serum bile acids	Normal	Normal
Appearance of liver	Grossly black; coarse, dark centrilobular pigment	Normal
Physical findings	Jaundice ± hepatomegaly	Jaundice
Urine coproporphyrins	Normal total; >80% isomer I	Markedly increased total; isomer I increased, but always <80%

Inheritance	Autosomal recessive	Autosomal recessive

Frequency

Dubin-Johnson syndrome has been described worldwide in all races, nationalities, and ethnic backgrounds and in both sexes (190,191,210,211,212,213,214). Uncommon on a worldwide basis, the disorder is highly prevalent (1:1,300) among Iranian Jews (191). In this population, its frequent association with a deficiency of clotting factor VII (215,216) appears to represent the coincidental inheritance of the two conditions, because factor VII deficiency is not observed as part of the syndrome in other populations, and is caused by a different genetic mutation (217,218).

Laboratory findings

Serum bilirubin concentration is typically between 2 and *5* mg/dL, but values as high as 20 or even 25 mg/dL have been reported (190,191). Fifty percent or more of total serum bilirubin is direct reacting, and accordingly, bilirubinuria and an increase in the covalently bound bilirubin fraction in plasma are frequently present. The serum bilirubin concentration often fluctuates, and occasional bilirubin determinations may be within normal limits. The results of other routine tests of liver function, including aminotransferases, alkaline phosphatase, and γ-glutamyltransferase activities; serum albumin concentration; cholesterol level; and (except in the Iranian population) prothrombin time are normal. Hematologic studies, including complete blood count, reticulocyte count, and red cell survival studies, are also normal (219), indicating no evidence of hemolysis (18,35,220). Fasting and postprandial levels of the common serum bile acids are normal in most patients with Dubin-Johnson syndrome (192,221), although mild elevations have been described in occasional patients (222).

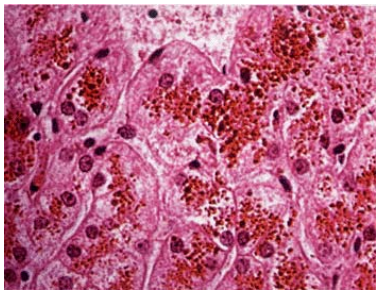
Imaging studies

Cholecystography, even when carried out with supplemental doses of contrast media, does not visualize the gallbladder in Dubin-Johnson syndrome (190,191), but visualization of the gallbladder is sometimes possible 4 to 6 hours after the intravenous administration of iodipamide (220,223). Biliary scintigraphy with agents such as Tc 99m Lidofenin or Tc 99m Disofenin may be helpful in the evaluation of patients with Dubin-Johnson and Rotor's syndromes (224,225,226). In six patients with Dubin-Johnson syndrome, administration of Tc 99m Lidofenin was followed by rapid, intense, homogeneous accumulation of the isotope within the liver without visualization of the intrahepatic biliary tree (224). In most patients with intact gallbladders, this organ was visualized approximately 90 minutes after the injection; in all cases, isotopic activity had reached the intestine within 1 hour of injection. However, in a patient with Rotor's syndrome, as well as in patients with jaundice and hepatocellular disease, administration of Tc 99m Lidofenin resulted in no visualization of the liver, gallbladder, or biliary tract and no accumulation of radionuclide in the intestine over 24 hours of observation. The kidneys visualized intensely in these latter conditions, indicating selective excretion of the radionuclide by this route (224).

Histopathology

Gross examination of the liver from typical patients with Dubin-Johnson syndrome shows it to be intensely pigmented to the point of appearing black in color (189,190). Light microscopy reveals no scarring, hepatocellular necrosis, or distortion of zonal architecture. Instead, the characteristic feature is the accumulation of a coarsely granular pigment, most pronounced in the centrilobular zones (Fig. 8.11) and with a characteristic lysosomal distribution (227). The nature of the pigment has been the subject of some debate, with some authors considering it a lipofuscin and others a melanin derivative. The histologically similar pigment observed

in mutant Corriedale sheep resembles melanin histochemically and incorporates tritium after the infusion of ^{3}H-epinephrine, a finding consistent with a melanin-like origin of the pigment granules (228). A study of Dubin-Johnson syndrome employing electron spin resonance spectroscopy demonstrated differences in the physicochemical characteristics of the Dubin-Johnson pigment when compared with authentic melanin (229). Although the nature of the pigment was not clearly defined, the data were consistent with its being composed of polymers of epinephrine metabolites. The degree of hepatic pigmentation may be variable, both within families and in a single individual with the Dubin-Johnson syndrome. Some of the variability may be genetic, but some may be due to the fact that coincidental diseases, such as viral hepatitis, are associated with the complete disappearance of the pigment from the liver (230). The pigment reaccumulates slowly after recovery from the hepatitic episode (231).

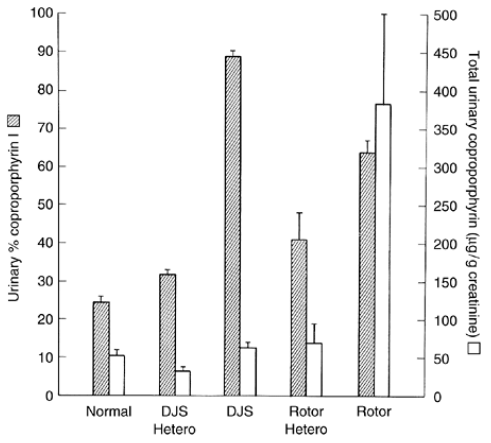


▪ **Figure 8.11** Liver biopsy in Dubin-Johnson syndrome showing coarsely granular pigment most pronounced in the centrilobular region (hematoxylin and eosin, original ×240). (Photomicrograph courtesy of Dr. Kamal G. Ishak, Armed Forces Institute of Pathology.)

Metabolism of organic anions

After intravenous injection, the initial disappearance of organic anions, such as bilirubin, BSP, dibromosulphthalein (DBSP), and ICG, from the plasma is normal (232,233). However, characteristic of Dubin-Johnson syndrome is that the plasma concentration of BSP is higher at 90 minutes as compared to 45 minutes after injection because of reflux of conjugated BSP back into the circulation from the hepatocyte, where it has been conjugated with GSH (191,192,220). This secondary rise is not seen after the intravenous administration of DBSP or ICG, compounds that are excreted into bile by the hepatocyte without further metabolism (232,233). A secondary rise, representing the reflux of conjugated bilirubin, has

been noted after the intravenous administration of unconjugated bilirubin-IXa to patients with this syndrome (229,232). The defect in the excretion of conjugated BSP has been confirmed by direct studies of the plasma clearance of this metabolite (234), consistent with the presence of a selective abnormality in the bile canalicular excretion of conjugated organic anions with normal excretion of bile salts. Studies of BSP metabolism involving continuous infusion of dye support these findings because they show a marked reduction in the calculated secretory transport maximum (T_m) with a normal relative storage capacity (S) (191,192). Although the secondary rise of BSP levels observed after intravenous administration of a single 5 mg per kg dose is highly suggestive of the syndrome, it is not diagnostic and has occasionally been observed in other hepatobiliary disorders (235).



▪ **Figure 8.12** Urinary coproporphyrin in Dubin-Johnson and Rotor's syndromes. The shaded bars represent the percentage of total urinary coproporphyrin excreted as coproporphyrin I. The open bars represent total urinary coproporphyrin excretion; vertical bars represent ± standard error of the mean. Total urinary coproporphyrin excretion is normal to slightly increased in the Dublin-Johnson syndrome (DJS), with a markedly elevated proportion of coproporphyrin I (approximately 80%). Both variables are markedly elevated in Rotor's syndrome. Therefore, with respect to urinary coproporphyrin excretion, the two disorders are distinct. Results in obligate heterozygotes (DJS hetero, Rotor hetero) lie intermediate between the results in healthy individuals and in individuals manifesting the respective disorder. (Reprinted with permission from Berk PD, Wolkoff AW, Berlin NI. Inborn errors of bilirubin metabolism. *Med Clin North Am* 1975;59:803–816.)

Urinary coproporphyrin excretion

Patients with Dubin-Johnson syndrome also have a diagnostic abnormality in urinary coproporphyrin excretion (210,236,237,238). There are two naturally occurring coproporphyrin isomers, I and III. Normally, approximately 75% of the coproporphyrin in the urine is isomer III. In the urine from patients with Dubin-Johnson syndrome, the total coproporphyrin content is normal, but more than 80% is isomer I. Heterozygotes for the syndrome show an intermediate pattern (210,239). The molecular basis for this phenomenon is not yet known.

Animal models of Dubin-Johnson syndrome

The mutant Corriedale sheep was the first recognized animal model of Dubin-Johnson syndrome (197,198,199,200). These animals have grossly black livers and defects in organic anion and coproporphyrin excretion that are identical to patients with the syndrome. Subsequently, two mutant rat models of Dubin-Johnson syndrome were described. These are the TR^- rat strain, also known as *GY*, and EHBR strain (80,202,203,204,205,206,207,208,209). These mutant rat strains exhibit many of the characteristic phenotypic features of the Dubin-Johnson syndrome, including (a) autosomal recessively inherited conjugated hyperbilirubinemia and bilirubinuria, (b) defective biliary excretion of conjugated organic anions with normal bile acid excretion, and (c) normal total urinary coproporphyrin excretion with increased percentage of isomer I. Although these animals do not typically have increased hepatic pigmentation, studies in TR^- animals demonstrate accumulation of lysosomal pigment after infusion of epinephrine metabolites or after feeding a diet high in aromatic amino acids (240). A mutant strain of golden lion tamarins with a Dubin-Johnson phenotype has also been described (201).

Inheritance

The initial descriptions of the Dubin-Johnson syndrome indicated that it was an inheritable disorder (190), and its familial nature has been verified in many subsequent studies (161,211,214,216,220,241,242). Although it was thought to be inherited as an autosomal recessive trait, there was initially no means to detect heterozygous carriers (243). This subsequently became available through the determination of urinary coproporphyrin excretion (210,239). As noted in preceding text, there is a characteristic abnormality in the pattern of urinary coproporphyrin excretion in patients with Dubin-Johnson syndrome (210,236,237). Subsequent studies found that in obligate heterozygotes (e.g., phenotypically healthy parents and children of patients with Dubin-Johnson syndrome), this pattern was intermediate between healthy and affected individuals, consistent with autosomal recessive inheritance (210,239). The pathogenesis of abnormal urinary coproporphyrin excretion in Dubin-Johnson syndrome is not known (244,245).

Genetic defect

The gene responsible for causing Dubin-Johnson syndrome has been identified as *MRP2*, encoding an ATP-dependent canalicular plasma membrane transporter for bilirubin conjugates and a number of other non–bile acid organic anions (246). Mutations in this gene can result in a Dubin-Johnson syndrome phenotype in rats and humans (214,246,247,248,249). Although it is clear that MRP2 plays a major role in the biliary excretion of conjugated bilirubin, even in its absence, bilirubin conjugates are found in bile, implying the existence of other, not yet identified, alternative transport proteins. At least 15 mutations in the MRP2 gene have been described in patients with the Dubin-Johnson syndrome (248). These include four splice site mutations, six missense mutations, three nonsense mutations, and one deletion mutation. As noted in preceding text, as many as 60% of patients with Dubin-Johnson syndrome in an Israeli series had coexistence of clotting factor VII deficiency. It is now known that the gene for factor VII is located on chromosome 13, in contrast to the localization of MRP2 to chromosome 10, which effectively excluded a primary genetic linkage between these two phenotypes.

Treatment

No specific therapy is indicated for Dubin-Johnson syndrome. It is generally considered to be a benign disease requiring only reassurance of the patient and avoidance of invasive diagnostic procedures. Although phenobarbital has been used in an attempt to reduce the serum bilirubin concentration, the results have been highly variable (250,251). Some patients with nonspecific abdominal complaints report amelioration of their vague symptoms during phenobarbital therapy, irrespective of any lowering of the serum bilirubin concentration; however, chronic phenobarbital administration is not recommended.

Rotor's syndrome

Rotor's syndrome characterized by chronic predominantly conjugated hyperbilirubinemia, phenotypically resembling the Dubin-Johnson syndrome (Table 8.4), was initially described in two Philippine families by Rotor et al. in 1948 (184). Although the incidence of Rotor's syndrome remains much less than that of the Dubin-Johnson syndrome, it has a widespread geographic distribution (252,253,254,255).

Clinical and laboratory findings

Patients with Rotor's syndrome are generally asymptomatic, and their conjugated hyperbilirubinemia is most often discovered incidentally. Some individuals describe mild symptoms such as weakness and vague abdominal pain, but pruritus is not a feature. In contrast to the Dubin-Johnson syndrome, hepatosplenomegaly has not been reported. The serum bilirubin concentration is typically elevated to between 2 and 5 mg/dL but may be as high as 20 mg/dL. More than half of the serum bilirubin is direct reacting, and bilirubinuria is typically present. Bilirubin levels often fluctuate in a given individual and may be increased by intercurrent illness. The results of conventional hepatic biochemical tests other than serum bilirubin, such as alkaline phosphatase and aminotransferase activities, serum albumin concentration, and prothrombin time, are typically normal (184,254). The gallbladder usually visualizes on oral cholecystography, in contrast to findings in the Dubin-Johnson syndrome (252,254). Liver biopsy is also normal, and there is no increase in hepatic pigmentation.

Organic anion metabolism

Although Rotor's syndrome phenotypically resembles the Dubin-Johnson syndrome and both disorders were initially considered to be variants of the same entity, detailed studies of BSP and coproporphyrin metabolism have indicated that they are distinct disorders (252,256). In addition, whereas oral cholecystographic agents usually do not visualize the gallbladder in the Dubin-Johnson syndrome, visualization is usual in Rotor's syndrome (254,257). After the administration of a 5-mg per kg dose of BSP, the initial plasma disappearance rate in Rotor's syndrome is markedly reduced and the 45-minute retention is elevated, often to 30% to 50% (256). There is no secondary rise in

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plasma BSP concentration, and conjugated BSP does not accumulate in plasma (256). Studies of biliary excretion failed to document a defect in the transport of either the free BSP or the BSP glutathione conjugate (234). In contrast to Dubin-Johnson syndrome, there is marked retention of ICG and unconjugated bilirubin after intravenous infusion (258). In addition, during constant infusion, the T_m for BSP in Rotor's syndrome was only minimally to moderately reduced, while the relative *S* was reduced by 75% to 90% (256,258). These data are in marked contrast to those in the Dubin-Johnson syndrome in which T_m is virtually 0, while *S* is normal. These results of T_m and *S* for BSP are identical to those that have been described in a phenotypically disorder that had been termed *hepatic storage disease* (185,259). It must be concluded that these two disorders are the same. When plasma BSP disappearance studies were conducted in 11 phenotypically normal obligate heterozygotes for Rotor's syndrome, mildly elevated retention at 45 minutes, averaging 11%, was intermediate between results in patients and healthy controls (256) Similarly, during constant BSP infusion studies, T_m and *S* in obligate heterozygotes were also intermediate between those of patients with Rotor's syndrome and healthy controls (256). These findings, suggesting an autosomal recessive mode of inheritance for Rotor's syndrome, are in contradistinction to similar studies in Dubin-Johnson syndrome, in which the carrier state is not usually detectable by studies of BSP metabolism.

Urinary coproporphyrin excretion

Rotor's syndrome is also differentiated from Dubin-Johnson syndrome with respect to patterns of urinary coproporphyrin excretion (238,252,260). Total urinary coproporphyrin excretion in patients with Rotor's syndrome is increased by 2.5- to 5-fold compared with healthy controls (252,260). The proportion of coproporphyrin I is also increased to an average of 65%. These results are similar to those seen in a variety of acquired hepatobiliary disorders and sharply contrast with those observed in Dubin-Johnson syndrome (261). Obligate heterozygotes for Rotor's syndrome are phenotypically normal and have a coproporphyrin excretion pattern intermediate between that of Rotor's syndrome and healthy controls (252). On the basis of urinary coproporphyrin excretion studies, Rotor's syndrome appears to be inherited as an autosomal recessive characteristic and is clearly distinct from Dubin-Johnson syndrome (Fig. 8.12) (252,260).

Genetic defect

It is clear that Rotor's syndrome is an inheritable disorder distinct from Dubin-Johnson syndrome (252,256,258,260).

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However, the genetic defect has not yet been elucidated.

Table 8.5. Inheritable Disorders Characterized by Conjugated Hyperbilirubinemia

	Dubin-Johnson	Rotor's	PFIC1	BRIC	PFIC2	BRIC type 2	PFIC3
Gene	*ABCC2*	Unknown	*ATP8B1*	*ATP8B1*	*ABCB11*	*ABCB11*	*ABCB4*
Protein	MRP2	Unknown	FIC1	FIC1	BSEP	BSEP	MDR3
Cholestasis	No	No	Yes	Episodic	Yes	Yes	Yes
Serum γ GT	Normal	Normal	Normal	Normal	Normal	Normal	↑↑
Serum bile acids	Normal	Normal	↑↑	↑↑ during episodes	↑↑	↑↑	↑↑
Clinical features	Mild conjugated hyperbilirubinemia; otherwise normal liver function; dark pigment in liver; characteristic pattern of urinary coproporphyrins	Mild conjugated hyperbilirubinemia; otherwise normal liver function; liver without abnormal pigmentation	Severe cholestasis beginning in childhood	Recurrent episodes of cholestasis beginning at any age	Severe cholestasis beginning in childhood	Recurrent episodes of cholestasis beginning at any age	Severe cholestasis beginning in childhood; decreased phospholipids in bile

PFIC, progressive familial intrahepatic cholestasis; BRIC, benign recurrent intrahepatic cholestasis; MRP2, multidrug resistance–associated protein 2; BSEP, bile salt export pump; MDR3, multidrug resistance protein 3; GT, glucuronosyltransferase.

Treatment

No treatment is required or effective for Rotor's syndrome. Most patients are in any case asymptomatic. In the absence of ill-advised medical or

surgical intervention, life expectancy appears to be normal.

Familial Cholestasis Syndromes

Several familial disorders characterized by conjugated hyperbilirubinemia in association with cholestasis have been described.

Benign Recurrent Intrahepatic Cholestasis

BRIC is a rare disorder characterized by recurrent attacks of pruritus and jaundice and typically presents with an episode of mild malaise, elevated serum aminotransferase levels, followed rapidly by rises in alkaline phosphatase and conjugated bilirubin (bilirubinuria) levels and onset of jaundice and itching (186,262,263,264). Initial episodes may be misdiagnosed as acute viral hepatitis. These episodes typically begin in childhood or young adulthood and can last for several weeks to months. Between attacks, lasting from several months to years, there is complete clinical and biochemical resolution. This disorder is familial and has an autosomal recessive pattern of inheritance. Although BRIC is considered a benign disorder that does not lead to cirrhosis or end-stage liver disease, the episodes can be quite debilitating, resulting in liver transplantation in some patients. Treatment during the cholestatic episodes is symptomatic and there is no means available to prevent or shorten the occurrence of episodes. A gene termed *FIC1* that encodes a protein FIC1, was recently identified and found to be mutated in most patients with BRIC (187). FIC1 is expressed primarily in the small intestine and weakly in the liver. It is a member of a P-type ATPase family that transports aminophospholipids from the outer to the inner leaflet of a variety of cell membranes and has little similarity to genes that have been shown to play a role in bile canalicular excretion of various compounds (187,265,266). How it causes BRIC remains unclear. Interestingly, a second phenotypically identical form of BRIC, termed *BRIC type 2*, has been described resulting from mutations in the bile salt export pump (BSEP), that resides normally on the bile canaliculus (267).

Progressive Familial Intrahepatic Cholestasis

The name PFIC is applied to three phenotypically related syndromes (Table 8.5). PFIC type 1 (Byler disease) presents in early infancy as cholestasis that may be initially episodic (268). However, in contrast to BRIC, Byler disease progresses to malnutrition, growth retardation, and end-stage liver disease during childhood. This disorder is also a consequence of a FIC1 mutation (187). The functional relationship of the *FIC1* protein to the pathogenesis of cholestasis in these disorders is unknown. Two other types of PFIC (types 2 and 3) have been described. Type 2 is associated with a mutation in the BSEP, the same protein that is abnormal in BRIC type 2 (269,270). Type 3 has been associated with a mutation of MDR3, a protein that is essential for normal hepatocellular excretion of phospholipids across the bile canaliculus (271,272). Although all three types of PFIC have similar clinical phenotypes, only type 3 is associated with high serum levels of γ-glutamyltransferase activity (264). In contrast, activity of this enzyme is normal or only mildly elevated in symptomatic BRIC and PFIC types 1 and 2.

Annotated References

Harris MJ, LE Couteur DG, Arias IM. Progressive familial intrahepatic cholestasis: genetic disorders of biliary transporters. *J Gastroenterol Hepatol* 2005;20:807–817.

Although the primary genetic lesions in the various forms of familial intrahepatic cholestasis do not cause defective bilirubin transport, they do lead to cholestasis and, secondarily, to conjugated hyperbilirubinemia. Studies of these genetic disorders have significantly helped unravel the basic mechanisms of the canalicular bile transport processes. This review covers the mechanism, clinical manifestations, genetics, and treatment of each disease.

Kadakol A, Ghosh SS, Sappal BS, et al. Genetic lesions of bilirubin uridine-diphosphoglucuronate glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype. *Hum Mutat* 2000;16:297–306.

This outstanding review evaluates the available information on the phenotypic consequences of multiple genotypes, including many mutations in both the UGT1A1 coding regions and the exon 1 promoter, which have been associated with Gilbert's syndrome and Crigler-Najjar syndromes type I and II. It produces a much clearer picture of the complex interactions among these three conditions.

Kapitulnik J. Bilirubin: an endogenous product of heme degradation with both cytotoxic and cytoprotective properties. *Mol Pharmacol* 2004;66:773–779.

Biliverdin, bilirubin, iron, and carbon monoxide, the biological products of heme degradation, have all been found to have potent biological effects. Furthermore, the rates of heme synthesis and degradation are tightly regulated. This has led to the proposal that heme turnover contributes, on the one hand, to pathogenesis of certain types of cellular injury and, on the other, to antioxidant defenses. This paper reviews this rapidly expanding field of research.

Ostrow JD, Pascolo L, Brites D, et al. Molecular basis of bilirubin-induced neurotoxicity. *Trends Mol Med* 2004;10:65–70.

The susceptibility of newborns to bilirubin neurotoxicity is highly variable. Furthermore, neurologic damage sometimes occurs at plasma unconjugated bilirubin (UCB) concentrations well below therapeutic

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guidelines. This paper reviews current information on mechanisms of cellular bilirubin toxicity, and the transport processes determining its access to and accumulation within the central nervous system.

Servedio V, d'Apolito M, Maiorano N, et al. Spectrum of UGT1A1 mutations in Crigler-Najjar (CN) syndrome patients: identification of twelve novel alleles and genotype-phenotype correlation. *Hum Mutat* 2005;25(3):325.

This brief paper, identifying 12 novel UGT1A1 mutations, reviewing the total spectrum of 77 such mutations identified as of early 2005, and exploring genotype–phenotype correlations is a valuable addition to the one by Kodakol et al listed above.

Takeuchi K, Kobayashi Y, Tamaki S, et al. Genetic polymorphisms of bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese patients with Crigler–Najjar syndrome or Gilbert's syndrome as well as in healthy Japanese subjects. *J Gastroenterol Hepatol* 2004;19:1023–1028.

This meticulous study analyzed both the UGT1A1 promoter and coding regions of 63 Japanese patients with Gilbert's syndrome and 71 healthy Japanese controls. The data indicate clearly that the genetic basis for the familial hyperbilirubinemias differs between Japanese and Caucasian populations.

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Chapter 9

Hepatic Histopathology

Zachary D. Goodman

Hala R. Makhlouf

Key Concepts

- Histopathologic examination of a liver biopsy specimen is a source of otherwise unobtainable qualitative information about the structural integrity of the liver tissue, the type and degree of injury, and the host's response to the injury; histopathologic examination also provides a basis for the diagnosis and classification of tumors.
- Histochemical and immunohistochemical stains are extremely helpful in evaluating the liver biopsy specimen.
- Qualitatively different patterns of injury can be used to distinguish diseases that have similar clinical presentations, such as chronic hepatitis, alcoholic or nonalcoholic steatohepatitis, and chronic cholestatic syndromes.
- Specific histologic features may allow precise diagnosis or strongly suggest a specific diagnosis. For example, “ground-glass” cells with positive histochemical or immunostaining for hepatitis B surface antigen indicates chronic hepatitis B infection, or florid duct lesions indicate the early stage of primary biliary cirrhosis.
- Accurate classification of tumors almost always requires histologic examination of tissue obtained by either biopsy or surgical excision.

The liver biopsy is an essential part of the investigation of diseases of the liver. Percutaneous (sometimes laparoscopic or transjugular) needle biopsies provide most of the specimens and the greatest challenge to the surgical pathologist, but open surgical biopsies and resection specimens obtained at laparotomy are also seen from time to time, especially when dealing with tumors, and even in total hepatectomies in

liver transplantation centers. Despite the many advances in laboratory tests, molecular diagnosis, and radiologic imaging techniques, liver biopsies continue to be performed. This is because histopathologic examination of the biopsy specimen is a source of otherwise unobtainable qualitative information about the structural integrity of the liver tissue, the type and degree of injury, and the host's response to the injury; histopathologic examination also provides a basis for the diagnosis and classification of tumors.

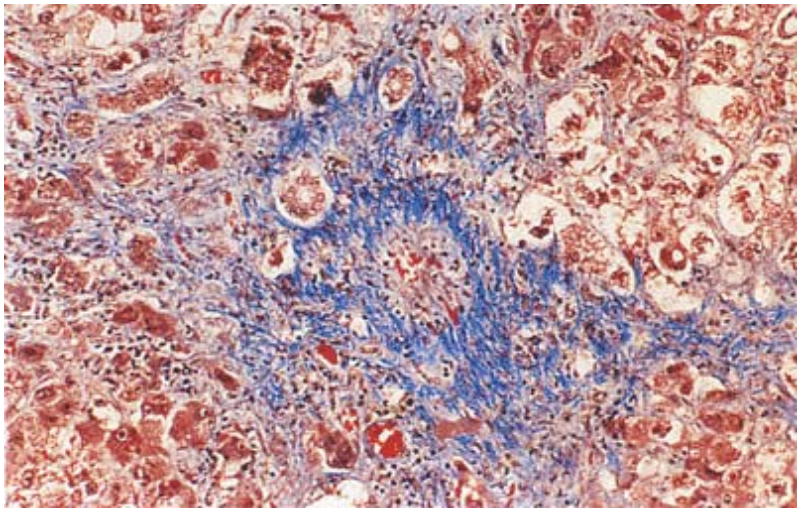
Liver biopsy should only be performed after a thorough noninvasive clinical evaluation. Furthermore, this information, including a history of possible exposure to hepatotoxins and sources of infection, pertinent physical findings, laboratory tests of liver function and integrity, serologic tests to detect infectious agents and autoimmunity, and radiologic studies, when appropriate, should be made available to the pathologist. Many biopsy specimens can be interpreted solely on morphologic grounds, and we recommend that the pathologist first examine the specimen and make initial observations without being biased by the clinical impression and laboratory data. However, after these initial observations, we strongly recommend that the

pathologist review the clinical and laboratory data, preferably with the clinician, and then arrive at the best possible clinical–pathologic correlation as the definitive diagnosis. This will allow the pathologist to avoid embarrassing errors that result from lack of information and will, at the same time, provide the clinician with an interpretation that will allow optimal patient care. In that regard, this chapter emphasizes the morphologic (predominantly light microscopic) aspects of the diseases of the liver, with clinical correlations in some of the major diseases.

Systematic Approach to the Liver Biopsy

The observer evaluating a liver biopsy specimen should always use a systematic approach to histopathologic evaluation. All fragments from a given biopsy specimen should be examined because a focal lesion, such as a granuloma, can be easily missed. In an architecturally normal liver it is best to begin evaluating by locating the terminal hepatic venules (or "central" veins) and then move in the direction of the portal areas. In doing so, changes involving the veins themselves and then the liver cells, bile canaliculi, abnormal deposits in spaces of Disse (e.g., collagen, amyloid), hypertrophied stellate cells, the sinusoids and their contents, and the Kupffer cells should be specifically looked for. The plates of hepatocytes nearest the portal areas should receive special attention, particularly in chronic necroinflammatory or cholestatic disorders. Proliferation of ductules and fibrosis also occur in this region of the acinus. All structures of the portal areas, namely, the connective tissue, bile ducts, veins, arteries, and lymphatics, as well as the inflammatory response (e.g., granulomas and the various types and

relative proportions of inflammatory cells), should be examined. In addition to searching for specific lesions, the absence of various structures in the portal areas such as the destruction and disappearance of bile ducts in primary biliary cirrhosis (PBC) or the obliteration of veins in hepatoportal sclerosis should also be carefully noted.



▪ **Figure 9.1** The Masson trichrome stains type I collagen blue, revealing zone 3 fibrosis in this case of alcoholic liver disease. An occluded terminal hepatic venule can be seen in the center of the field.

Special Histochemical Stains

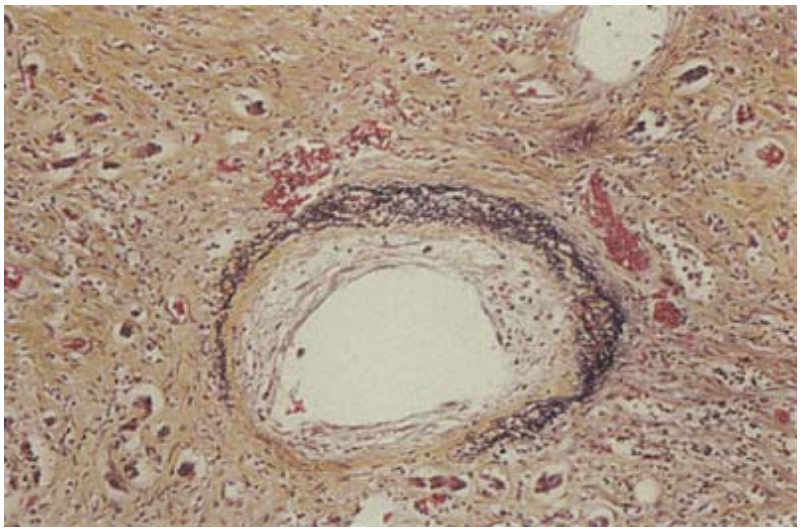
Although most of the observations that lead to a diagnosis are made using the routine hematoxylin and eosin stain, special stains are helpful in evaluating liver biopsy specimens. Staining methods referred to in this chapter can be found in the Armed Forces Institute of Pathology's *Laboratory Methods in Histotechnology* (1).

Fibrosis and connective tissue

Evaluation of the presence, extent, and location of fibrosis is essential in the diagnosis of non-neoplastic liver disease. A Masson trichrome (Fig. 9.1) is useful for demonstrating the degree of fibrosis and cirrhosis in chronic liver disease and is also useful in assessing changes involving arteries and veins, such as the lesions of veno-occlusive disease and hepatic vein thrombosis. A Movat pentachrome stain is particularly useful for vascular lesions because it stains elastica and

acid mucopolysaccharide, in addition to collagen and smooth muscle (Fig. 9.2). Elastic tissue can also be well demonstrated by orcein or

Victoria blue stains that are used to identify ground-glass cells containing hepatitis B surface antigen. A reticulin stain is useful for outlining areas of focal or zonal necrosis (Fig. 9.3), thick liver plates, or nodules of regeneration. It can also be used to demonstrate fibrosis, but in general the Masson stain is preferred because the reticulin stain does not distinguish permanent scarring from stromal collapse.

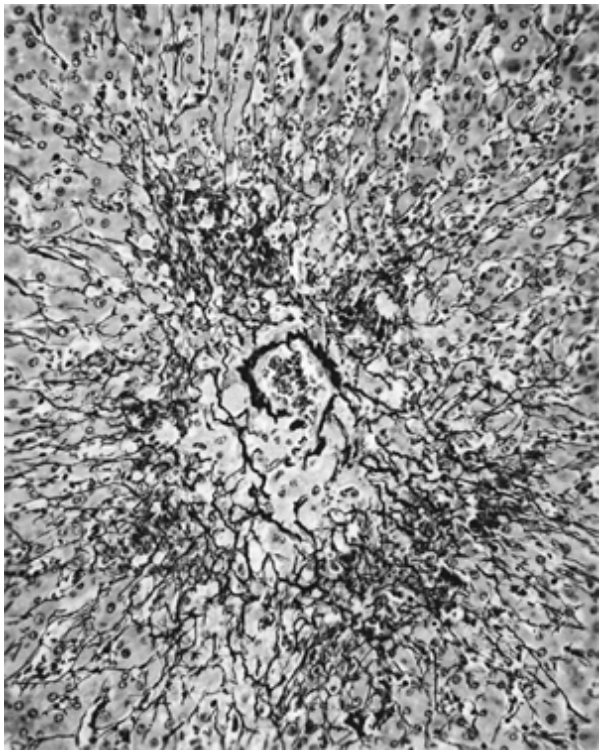


▪ **Figure 9.2** The Movat pentachrome stain shows a partially occluded outflow vein in this case of alcoholic cirrhosis. The elastic tissue in the wall of the vein is black, whereas mucopolysaccharides in the hypertrophied intima are pale blue, and collagen in the cirrhotic scars is yellow green.

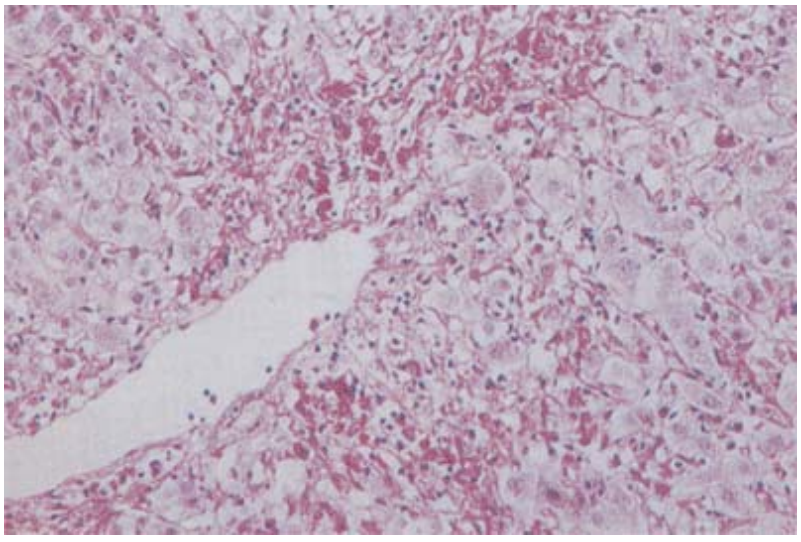
Complex carbohydrates

Complex carbohydrates are readily demonstrated with the periodic acid-Schiff (PAS) stain. This will of course demonstrate glycogen in liver cells or in benign and malignant hepatocellular tumors, but a much more useful stain that should be routinely employed is the PAS stain after pretreatment with diastase (DPAS) to remove the glycogen and unmask other complex carbohydrate–containing substances, including metabolic and synthetic products of the liver, and structural components. The DPAS stain strikingly demonstrates the presence of lipofuscin and other cell debris in Kupffer cells and portal macrophages in acute hepatocellular injury (Fig. 9.4). The Dubin-Johnson pigment in liver cells stains variably with PAS. In type IV glycogenosis, Lafora's disease (myoclonus epilepsy), and cyanamide-induced liver injury, hepatocytes (mainly in zone 1) contain PAS-positive material that resists diastase digestion, but in Lafora's disease and glycogenosis type IV the material can be digested by pectinase. The globules of α_1-

antitrypsin are strongly DPAS positive (Fig. 9.5). PAS is also useful for staining bile duct basement membranes, to demonstrate ductal injury; fibrin (e.g., in disseminated intravascular coagulation); amyloid; starch; amoebae; and most pathogenic fungi.



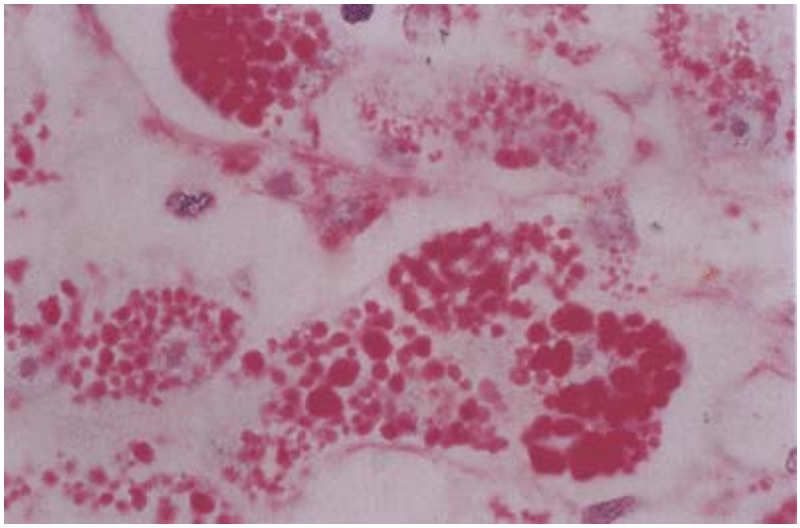
▪ **Figure 9.3** The reticulin stain shows the black-staining type III collagen fibers that support the liver cell plates. A terminal hepatic venule in the center of the field is surrounded by collapsed reticulin, indicating zone 3 necrosis.



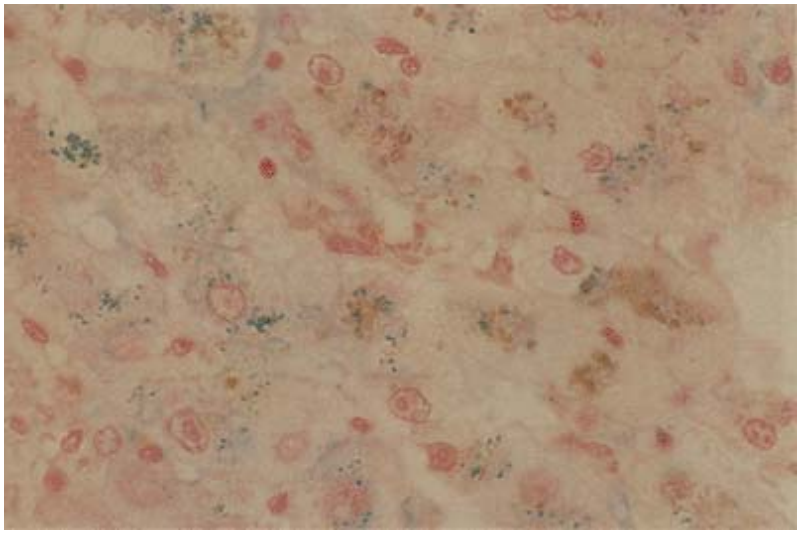
▪ **Figure 9.4** The periodic acid-Schiff stain after diastase digestion to remove glycogen demonstrates the presence of lipofuscin and other cell debris in Kupffer cells in acute hepatocellular injury.

Iron

Iron is readily demonstrated with the Prussian blue stain, which is also useful in bringing out the green color of bile and the golden brown color of lipofuscin, both of which can be masked by overstaining with either eosin or hematoxylin (Fig. 9.6). Copper is specifically best demonstrated by the rhodanine stain (Fig. 9.7), but the orcein and Victoria blue stains, which are technically easier, can also be used because these will stain concentrated deposits of the copper-binding protein, metallothionine. In the past, orcein and Victoria blue were primarily used to demonstrate the ground-glass inclusions of chronic hepatitis B infections (Fig. 9.8), but these have now been largely supplanted by specific immunostains, as discussed later.

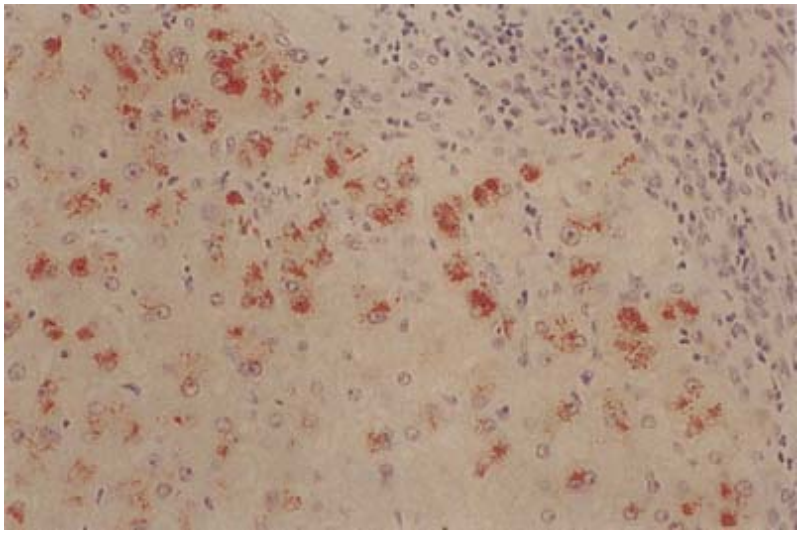


▪ **Figure 9.5** Globules of α_1-antitrypsin are strongly periodic acid-Schiff positive and diastase resistant.



▪ **Figure 9.6** The Prussian blue stain for iron demonstrates hemosiderin (blue granules) and also brings out the green color of bile and the golden brown color of lipofuscin.

Other stains that find occasional use include the Hall's stain for bile; the Fontana's stain for lipofuscin and Dubin-Johnson pigment; the phosphotungstic acid–hematoxylin stain for fibrin or mitochondria; the Congo red, sirius red, or crystal violet stains for amyloid; an acid-fast stain for mycobacteria, schistosome eggs, or the hooklets in the scolices of echinococcal cysts; and the Warthin-Starry stain for spirochetes, leptospira, or the bacilli causing catscratch disease. A Giemsa stain can be useful in studying the morphology of hematopoietic cells or in identifying some microorganisms, such as *Leishmania* or *Cryptosporidia*.

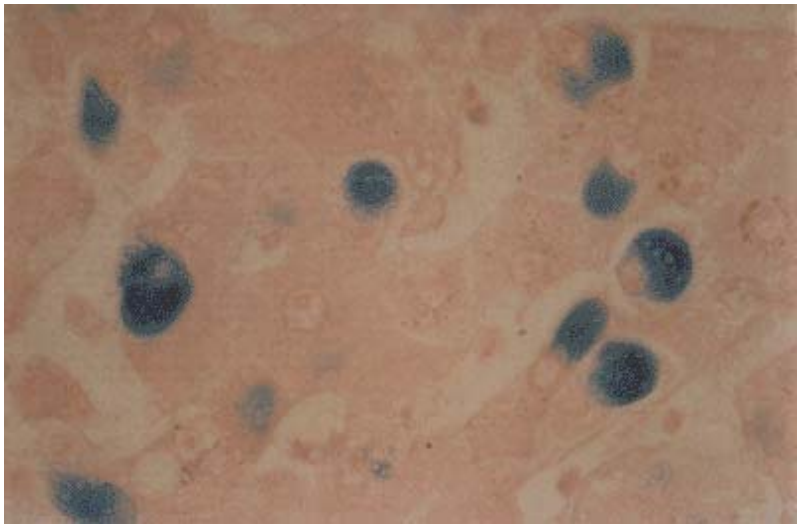


▪ **Figure 9.7** The rhodanine stain demonstrates copper as brick-red granules in liver cells in this case of Wilson disease.

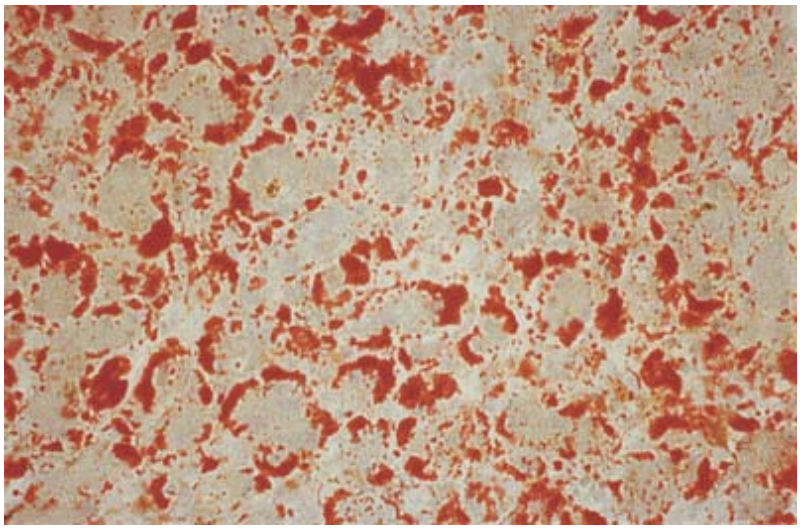
Fat

Fat can be demonstrated histochemically, but this requires unprocessed frozen sections, which must be prepared with a cryostat from fresh or formalin-fixed

tissue. Routine processing exposes the tissue to organic solvents, which will extract lipids and render fat stains useless. However, frozen sections prepared and stained with oil red-O (Fig. 9.9) can be quite useful for demonstrating neutral lipid in liver cells in a variety of conditions or in cells of benign or malignant tumors. This stain may also be used to demonstrate fat globules of stellate cells, cholesterol crystals (which are also birefringent when examined under polarized light), and lipofuscin in liver or Kupffer cells. Cholesterol can be specifically stained in frozen sections by the Schultz modification of the Liebermann-Burchard reaction, a reaction useful for the diagnosis of Wolman's disease or cholesteryl ester storage disease. Metachromatic granules in macrophage cells and bile duct epithelium in metachromatic leukodystrophy are best demonstrated in frozen sections by using stains such as cresyl violet or toluidine blue.



▪ **Figure 9.8** The Victoria blue stain shows cells containing large amounts of hepatitis B surface antigen in a chronic carrier.



▪ **Figure 9.9** Oil red O stain of a frozen section shows microvesicular fat in acute fatty liver of pregnancy.

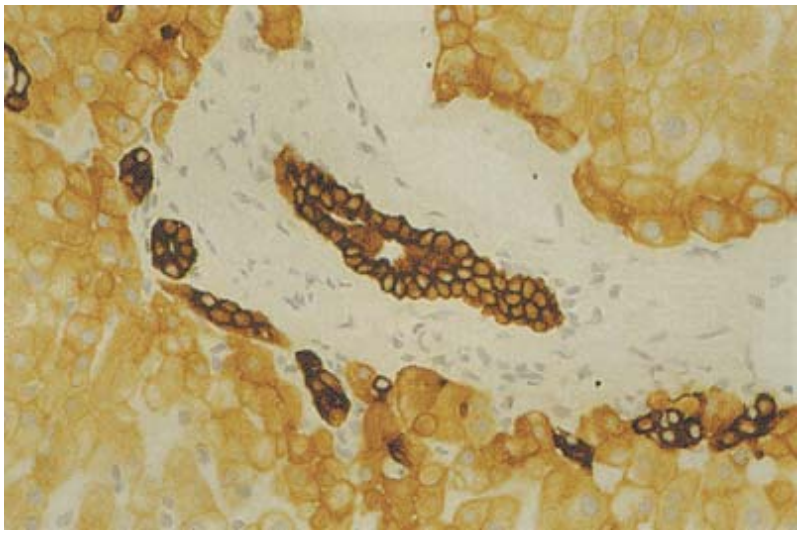
Immunopathology

Immunostains are now routinely utilized in the diagnosis of hepatic neoplasms, but it has fewer practical applications in the study of non-neoplastic diseases of the liver. Nevertheless, these techniques can be

used to demonstrate and characterize normal structural components and a number of histopathologic changes; they can also be used to locate viral antigens and other infectious agents.

In the normal liver, bile duct epithelial cells react with monoclonal antibodies to cytokeratins 7, 8, 18, and 19 (Fig. 9.10), whereas the liver cells react only with monoclonal antibodies to cytokeratins 8 and 18 (2). Bile canaliculi are demonstrated by polyclonal antibodies to carcinoembryonic antigen (CEA) (Fig. 9.11) because of the presence of a cross-reacting biliary glycoprotein. Tumors derived from hepatocytes and bile ducts often maintain the antigenic characteristics of their normal counterparts, but this is not invariable, hence staining patterns must be interpreted in the context of other histologic features.

A few practical applications for immunohistochemistry have been found in the diagnosis of non-neoplastic diseases. In chronic cholestatic disorders, such as PBC, cytokeratin type 7, normally only found in biliary-type cells, appears in periportal hepatocytes (3). Because cytokeratin proteins are a major component of Mallory bodies, antibodies to several high- and low-molecular-weight keratins may be used to demonstrate this pathologic feature, but none is as reliable for this purpose as staining with antibodies to ubiquitin (4), a cellular stress protein that coats the surface of filamentous tangles such as Mallory bodies (Fig. 9.12). Ubiquitin also coats tangles of amyloid filaments, but there are specific antibodies that can be used to confirm the diagnosis of amyloidosis and characterize the different types of amyloids (5).



▪ **Figure 9.10** Immunostain with a cocktail of monoclonal antibodies that react with cytokeratin types 7, 8, 18, and 19. There is strongly positive staining of the bile duct in the center of the portal area and the ductular cells at the edge, whereas hepatocytes

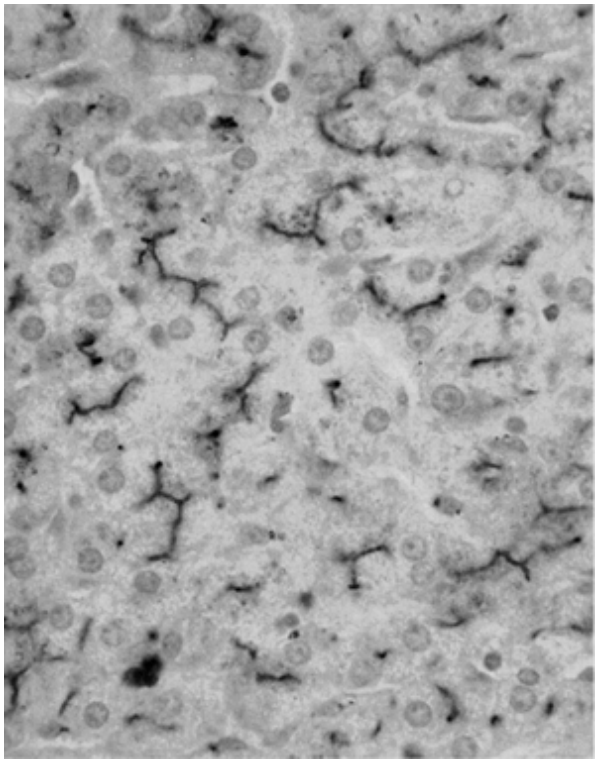
stain only weakly.

Immunostaining for viral antigens in different types of viral hepatitis is extensively used for investigational purposes and, to a lesser extent, for diagnosis. In routine practice, hepatitis B antigens (surface and core) are readily identified with commercially available antisera (Fig. 9.13). Hepatitis D can also be identified in routinely processed tissue, but antibodies are more difficult to obtain. Hepatitis A, C, and E can only be reliably identified in frozen sections, and staining for these is generally limited to research settings. Several viruses, other than those causing viral hepatitis, including herpes simplex virus, Cytomegalovirus (CMV), and adenovirus, can be detected in the liver by using commercially available antisera.

Electron Microscopy

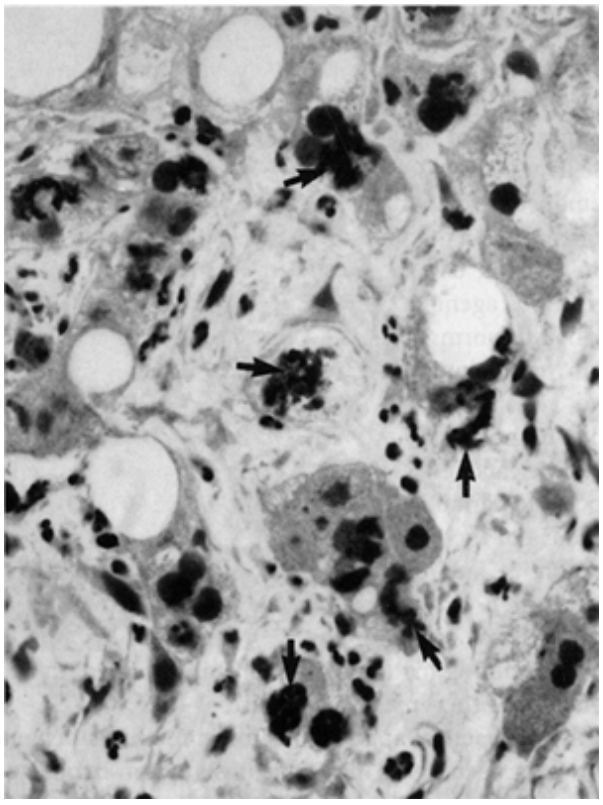
Transmission electron microscopy has many investigational but a limited number of diagnostic applications (6). Its greatest value is in the interpretation of biopsy specimens from patients with known or suspected metabolic disorders, and it can also be helpful in drug-induced and cholestatic diseases and in some infections. Among the metabolic diseases, distinctive or pathognomonic ultrastructural findings are present in hereditary fructose intolerance, α_1-antitrypsin

deficiency, Farber's disease, glycogenoses types II and IV, Gaucher's disease, metachromatic leukodystrophy, Dubin-Johnson syndrome, erythropoietic protoporphyria, Wilson disease, Zellweger syndrome, and many others. Drug-induced injury causes changes in many organelles of the liver depending on the drug, duration of use, and other factors. Many drugs (e.g., phenytoin, phenobarbital) and toxins (e.g., DDT [2,2-bis(*p*-chlorophenyl)-1,1,1,-trichloroethane] and other pesticides) cause proliferation of the smooth endoplasmic reticulum of liver cells ("induced" hepatocytes), which results in a characteristic ground-glass appearance on light microscopy. Megamitochondria, sometimes assuming monstrous forms, are considered typical of drug reactions, while lysosomal phospholipidosis is highly typical of several drugs (e.g., amiodarone). Subtle manifestations of cholestasis due to a variety of causes can be seen ultrastructurally before becoming recognizable by light microscopy. Among infectious agents, viral particles can be visualized directly in herpes simplex, adenovirus, and CMV infections, and both incomplete and complete particles of hepatitis B can be seen in infected liver cells.



▪ **Figure 9.11** Immunostain with polyclonal antibodies to carcinoembryonic antigen demonstrates dark-staining bile canaliculi between hepatocytes.

Scanning electron microscopy has also proved more useful for investigation than for diagnosis. The diagnostic applications of this technique are largely limited to particulate material, especially when x-ray spectrophotometry (also called *electron probe analysis*) is combined with scanning electron microscopy (7). Using this technique, the elements that are present in particulate material, such as talc, Thorotrast, silicone, silica, titanium, gold, and barium sulfate can be positively identified.



▪ **Figure 9.12** Immunostain with antibody to ubiquitin demonstrates dark-staining Mallory bodies (*arrows*) in liver cells in alcoholic hepatitis.

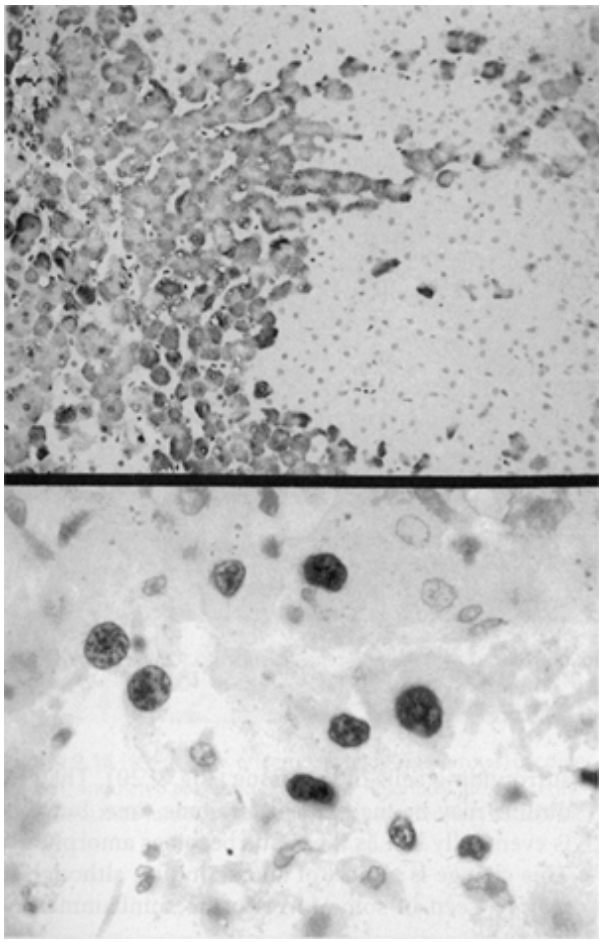
Other Special Techniques

In situ hybridization is used to detect genomic deoxyribonucleic acid (DNA) of the hepatitis B virus (HBV) (8), and there are reports of the use of in situ hybridization and in situ polymerase chain reaction (PCR) to demonstrate the presence of hepatitis C virus (9), but currently, these are essentially research techniques.

Polarizing microscopy is useful in identifying birefringent crystals of talc (Fig. 9.14) in portal macrophages or Kupffer cells in abusers of intravenous drugs (10). The remnants of previous surgery, such as suture material, talc, or starch from glove powder left on the surface of the liver are also birefringent in polarized light, as is silica in multiorgan silicosis (11). Type I collagen has a silvery birefringence, and amyloid has a characteristic apple green birefringence when sections stained with Congo red are examined by polarizing microscopy. Formalin pigment (typically in

blood vessels) and both malarial and schistosomal pigments (in

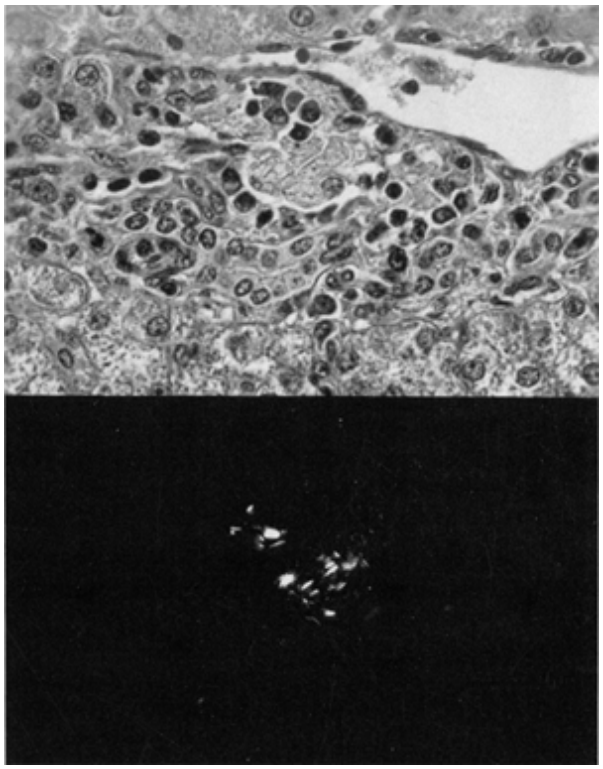
reticuloendothelial cells) are brown to black deposits of acid hematin and are birefringent under polarized light. Cholesterol crystals (e.g., in the livers of patients with Wolman's disease and cholesteryl ester storage disease) in frozen sections, whether stained or unstained, are birefringent, as are cystine crystals in cystinosis. Needle-like uroporphyrin crystals in liver cells can sometimes be visualized by polarizing microscopy of unstained frozen or paraffin sections in porphyria cutanea tarda (12). Red birefringent Maltese crosses and amorphous materials are characteristic of protoporphyrin accumulation in canaliculi or Kupffer cells in erythropoietic protoporphyria (Fig. 9.15) (13).



▪ **Figure 9.13** Immunostains for hepatitis B antigens. Antibody to HBsAg (*top*) shows variable amounts of the antigen in the cytoplasm of some hepatocytes. Antibody to HBcAg (*bottom*) shows the antigen in nuclei of liver cells with replicative virus.

Ultraviolet (UV) microscopy is most useful in confirming the diagnosis in the hepatic porphyrias. However, unfixed, air-dried frozen sections are required, so the usefulness of the technique is limited. Nevertheless,

frozen sections of the liver in porphyria cutanea tarda and erythropoietic protophorphyria reveal red autofluorescence under UV microscopy because of the presence of porphyrins. Vitamin A stored in stellate cells has a green and rapidly fading autofluorescence in UV light, while a granular yellow autofluorescence is characteristic of lipofuscin.



▪ **Figure 9.14** Portal macrophages in the center of field (*top*) contain talc crystals, which are birefringent and easily visualized with polarizing microscopy (*bottom*).

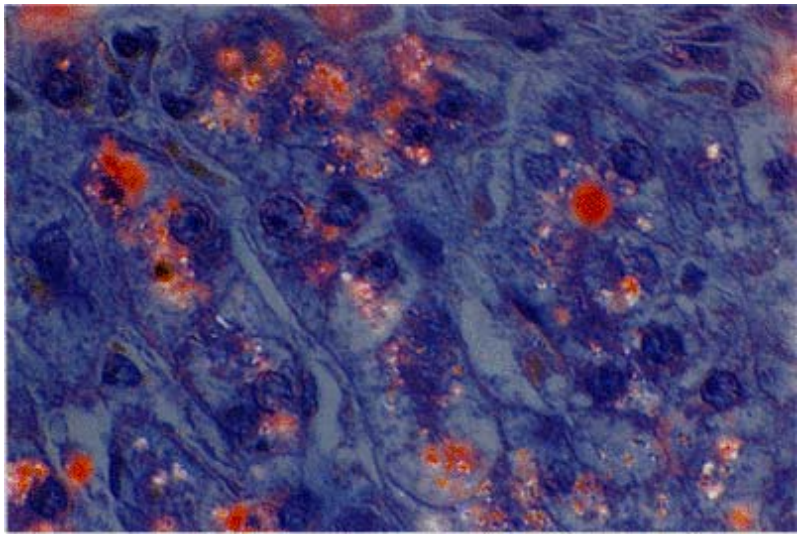
Morphologic Patterns of Injury

Acute Necroinflammatory Disease (Acute Hepatitis)

Acute necroinflammatory disease is typically seen in case of acute infections with the hepatitis viruses, but identical injury may occur with hepatitis-like reactions to a number of therapeutic drugs (see Chapter 33). Hepatocellular injury, leading to cell death, is the predominant morphologic feature of acute necroinflammatory diseases, although the term *necroinflammatory* has become something of a misnomer in view of recent advances in pathobiology. The term *necrosis*, previously used for all forms of cell death, is now applied more selectively to certain

forms of cell death. Many of the injured and dying cells seen in the various forms of hepatitis are actually in the process of apoptosis, while the "inflammatory" component is at times the effector of

apoptosis and at times the response to the hepatocellular injury. Nevertheless, for the purposes of this discussion, the term *necroinflammatory* will be maintained.



▪ **Figure 9.15** Deposits of protoporphyrin are birefringent and appear red in polarized light. Larger deposits show a characteristic Maltese cross.

Several basic lesions are seen in various forms of necroinflammatory injury:

1. *Apoptosis* (14, 15) results in the shrinkage of the hepatocyte, which often has an angular configuration and is more eosinophilic than its neighbors in the liver plate ("acidophilic degeneration") (Fig. 9.16). The nucleus is often pyknotic and deeply basophilic. The cytoplasm of the liver cell develops protuberances that separate and are released into spaces of Disse and sinusoids. The larger cell fragments, which may contain parts of the nucleus, have been termed *apoptotic, acidophilic*, or *hyaline* bodies (Figs. 9.17 and 9.18). The apoptotic bodies are quickly phagocytosed by Kupffer cells or adjoining liver cells, where they undergo degeneration and are reduced to residual bodies.
2. *Ballooning degeneration* refers to the swelling of hepatocytes, often to several times the normal size. Affected cells have an indistinct cell membrane, and the cytoplasm is rarefied (Figs. 9.18

and 9.19). The ballooned hepatocytes eventually undergo lysis, with disappearance or "dropping out." The remnants of these cells attract lymphocytes and, less often, other types of inflammatory cells ("focal necrosis"), as well as hypertrophied Kupffer cells.

3. *Coagulative necrosis* refers to a form of cell death recognized by deeply eosinophilic, granular cytoplasm with loss of the nucleus and discohesion from the surrounding cells of the tissue (Fig. 9.20). The cell outline may be maintained for some time, but this is eventually lost as the tissue becomes amorphous. This change is typical of anoxic injury, although it may be seen in some forms of necroinflammatory injury.
4. *Regeneration*, recognized by enlargement of nuclei and nucleoli, mitoses, binucleation, and thickening of liver cell plates (Fig. 9.21), may be seen shortly after the onset of a necroinflammatory injury such as viral hepatitis. The number of regenerating cells gradually increases as the patient recovers.
5. *Kupffer cell hypertrophy* is characteristic of acute necroinflammatory injuries. Sinusoidal macrophages are normally inconspicuous, but in response to liver cell death, these enlarge as they perform their phagocytic function, and they can be recognized by the presence of cytoplasmic light brown, finely granular lipofuscin presumed to be phagocytosed from necrotic hepatocytes (Figs. 9.21 and 9.22).

Acute necroinflammatory patterns

"Classic" acute hepatitis, typical of the common forms of acute viral hepatitis is characterized by panacinar necroinflammatory disease with spotty necrosis. The appearance of the liver is one of acinar disarray (Fig. 9.23) caused by widespread degeneration and death of individual and small groups of hepatocytes, which display features of both apoptosis and ballooning with focal necrosis. These are seen throughout the acinus in various combinations, and not all hepatocytes in a given acinus

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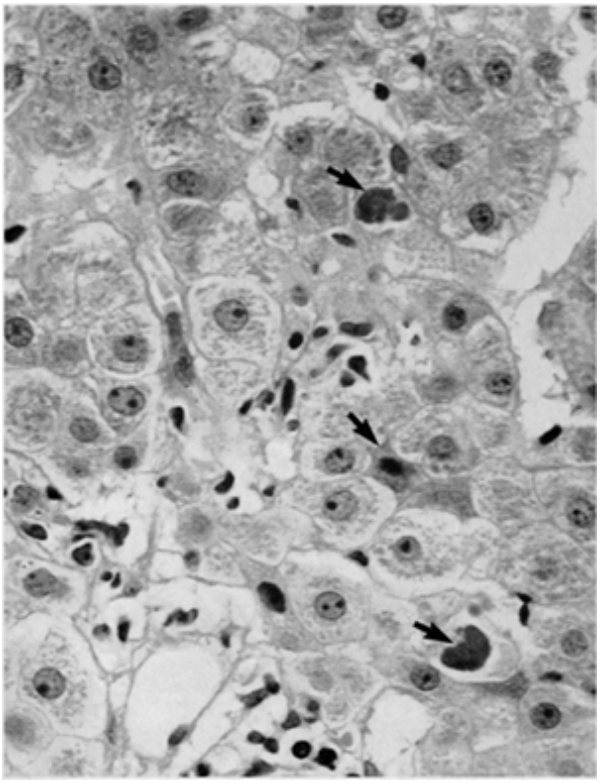
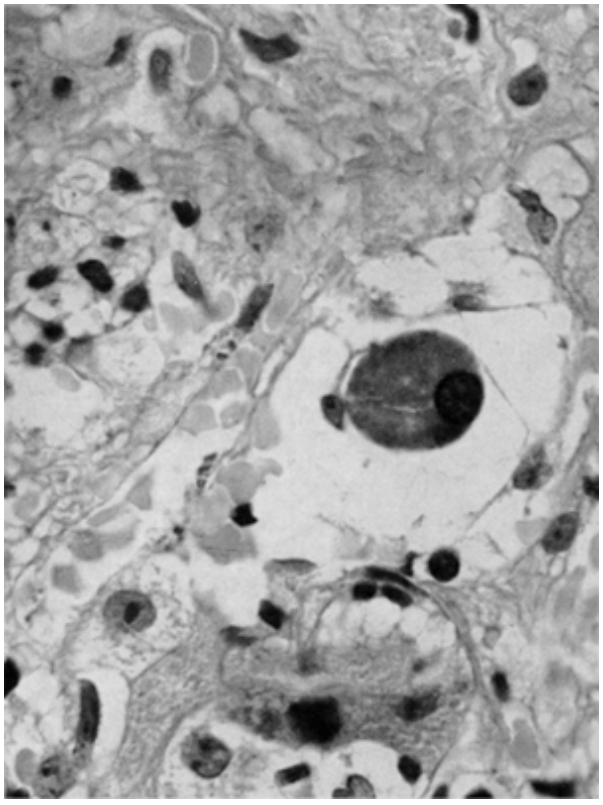
are affected. Features of regeneration are invariably present, and there is typically an inflammatory response consisting of hypertrophied Kupffer cells and lymphocytes. The portal areas in typical acute hepatitis are usually infiltrated with inflammatory cells. Lymphocytes predominate, but a small number of plasma cells, as well as eosinophils and neutrophils (especially in drug-induced disease), may be present. Occasionally, plasma cells predominate, especially in hepatitis A (16,17). The inflammatory response often extends beyond the confines

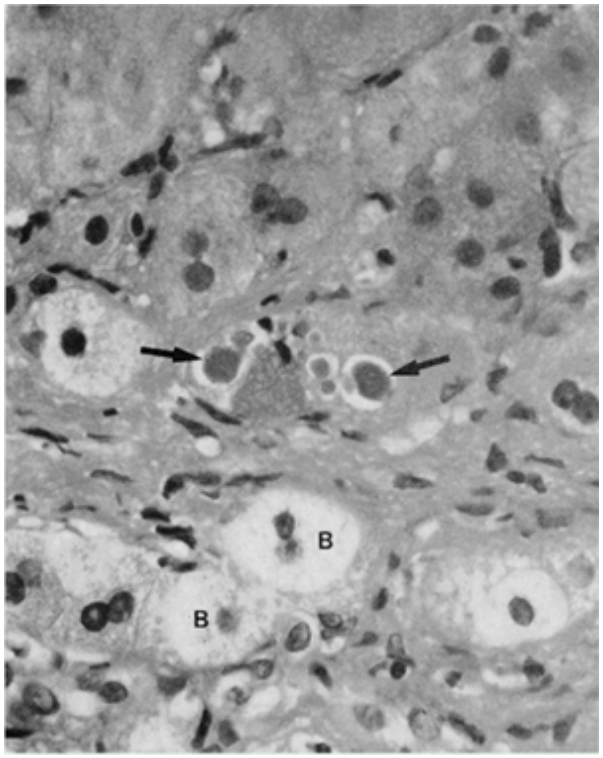
of the portal areas, leading to some blurring of the outline of the limiting plate and creating the appearance of interface hepatitis, as discussed later under chronic hepatitis. This also is especially true in hepatitis A (Fig. 9.24). Cholestasis is not a significant component of the histopathology of "classic" acute viral hepatitis, and when present, it is usually seen as an occasional, haphazardly distributed canalicular bile plug. Occasional cases may reveal abnormalities of the bile ductal epithelium, including swelling, disruption, and infiltration by lymphocytes, which constitute the hepatitis-associated bile duct lesions that are discussed in the section on chronic hepatitis.



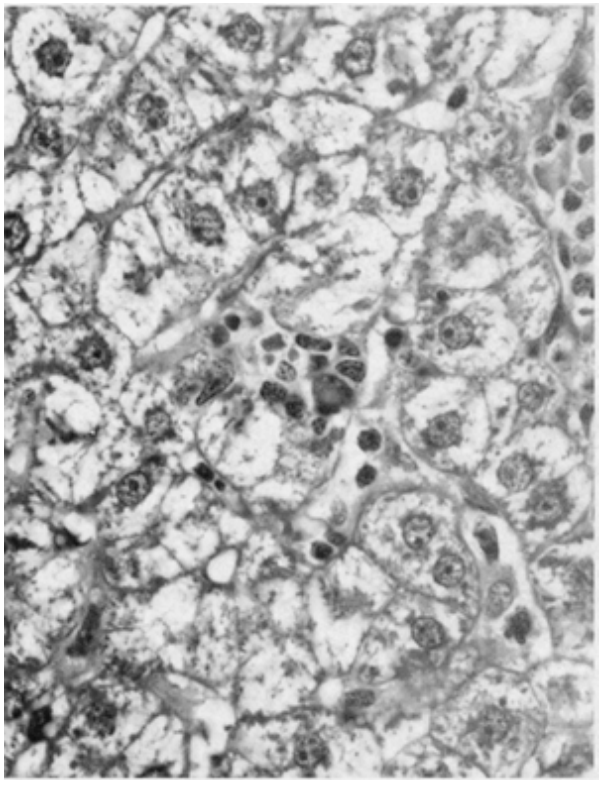
▪ **Figure 9.16** Hepatocytes undergoing apoptosis (*arrows*) become shrunken, angulated and darker than their neighbors (*arrow*, center of field), lose their nuclei and begin to fragment, forming acidophilic bodies.



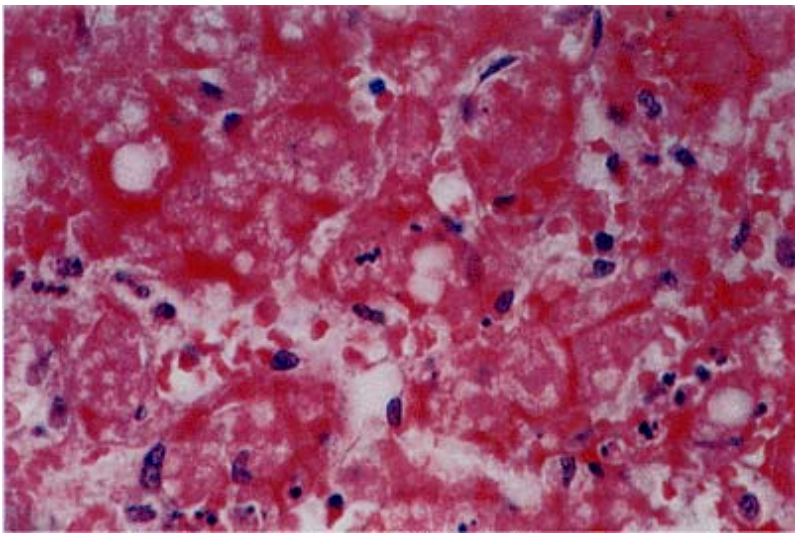
▪ **Figure 9.17** High magnification of an acidophilic body that has been extruded from the liver cell plate into a sinusoid. Most of the degenerated nucleus of the dead hepatocyte remains.



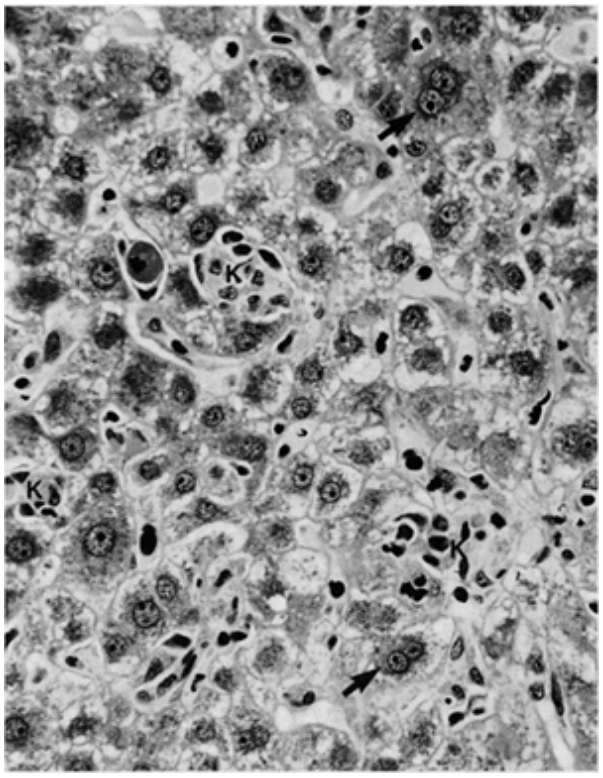
▪ **Figure 9.18** Cellular degeneration and death with apoptotic bodies (*arrows*), and ballooning (*B*).



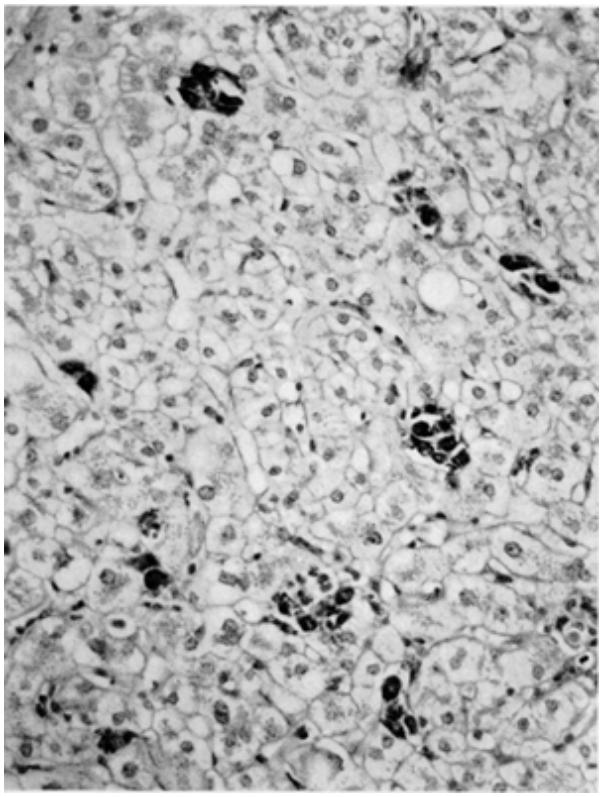
▪ **Figure 9.19** Ballooning degeneration. The hepatocytes are swollen and pale. A cluster of inflammatory cells ("focal necrosis") in the center of the field shows the position where a hepatocyte has disappeared from the tissue.



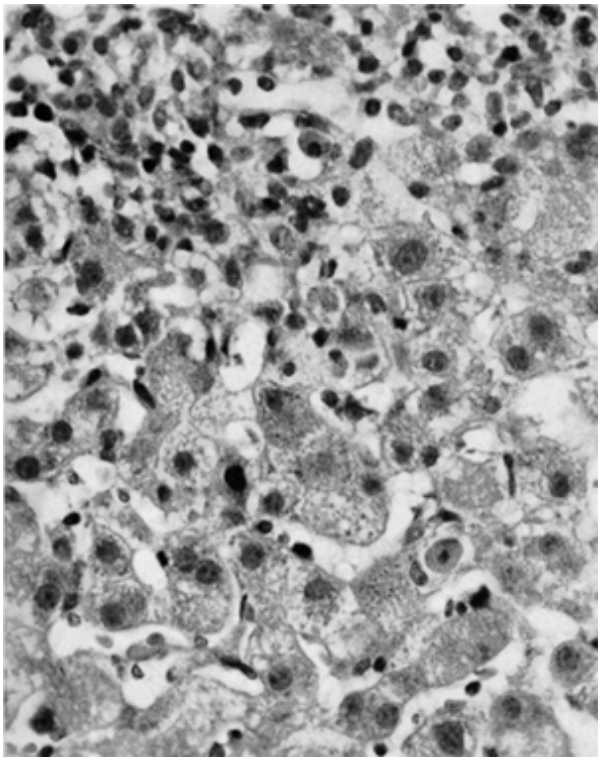
▪ **Figure 9.20** Coagulative necrosis in a case of ischemic injury. The cytoplasm of the necrotic cells is eosinophilic and granular, and the nuclei have disappeared.



▪ **Figure 9.21** Active necroinflammatory injury with regenerating liver cells, recognizable by enlarged nuclei, prominent nucleoli, and binucleate liver cells (*arrows*). Clusters of hypertrophied Kupffer cells (*K*) are present at sites of liver cell dropout.

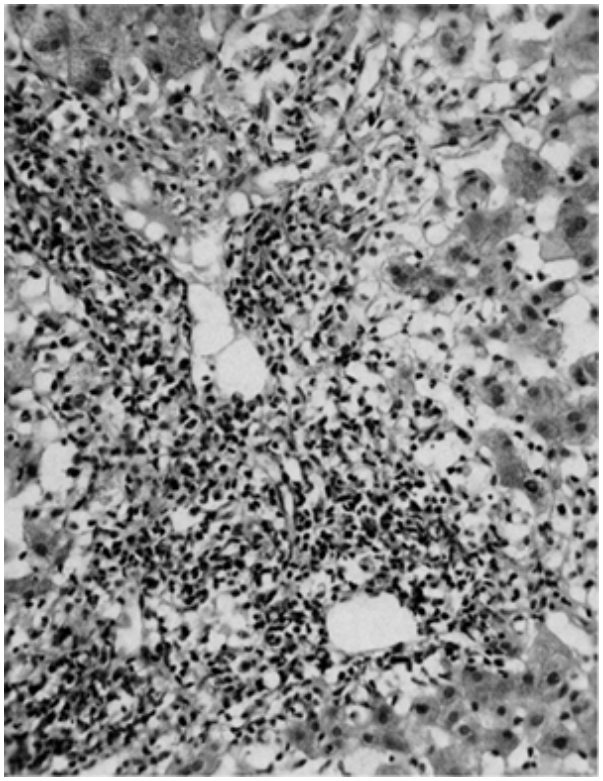


▪ **Figure 9.22** The periodic acid-Schiff stain after diastase digestion demonstrates clusters of hypertrophied, lipofuscin-filled Kupffer cells (dark-staining) at the sites of liver cell dropout.



▪ **Figure 9.23** Acute viral hepatitis. Note acinar disarray, apoptosis, and focal necrosis, Kupffer cell hypertrophy, and lymphocytic infiltrate.

The subsiding phase of viral hepatitis is characterized by a lessening of the injury and inflammation with increased regeneration and repair. The differences between this and the active phase are, however, mainly quantitative. Acinar disarray diminishes and eventually disappears, and the hepatic parenchyma gradually reverts to a normal appearance over a period of several weeks to months, although varying degrees of unrest are still evident. The liver cell plates often appear thickened. Only occasional degenerating cells and small foci of inflammation are evident. A frequent finding is the continuing hypertrophy of Kupffer cells and portal macrophages (Fig. 9.22). They become relatively more conspicuous because the hepatocytes are less swollen, and they now contain variable amounts of hemosiderin in addition to lipofuscin. The portal area inflammatory response gradually diminishes. Uncomplicated viral hepatitis is not followed by any significant periportal or intra-acinar fibrosis.

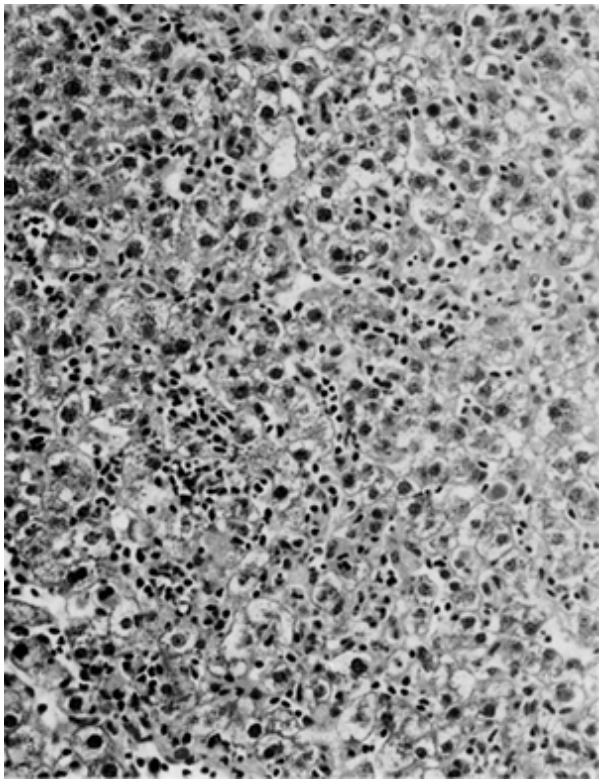


▪ **Figure 9.24** Acute hepatitis A, showing marked portal inflammation with extension into the adjacent parenchyma (interface hepatitis), mimicking the appearance of chronic hepatitis.

Mononucleosis hepatitis is typical of Epstein-Barr virus (EBV) and CMV infections in immunocompetant patients (18,19,20). Similar histology can be seen in reactions to some drugs, especially diphenylhydantoin (21), and we have also observed this reaction on occasion in acute hepatitis B or C, so a complete serologic workup is advisable whenever this pattern of injury is seen. In comparison with "classic" viral hepatitis, the inflammatory response in this variant is more prominent (Fig. 9.25) while the hepatocellular injury is milder. Hepatocellular regeneration is prominent, and mitotic figures are often seen in hepatocytes, Kupffer cells, and

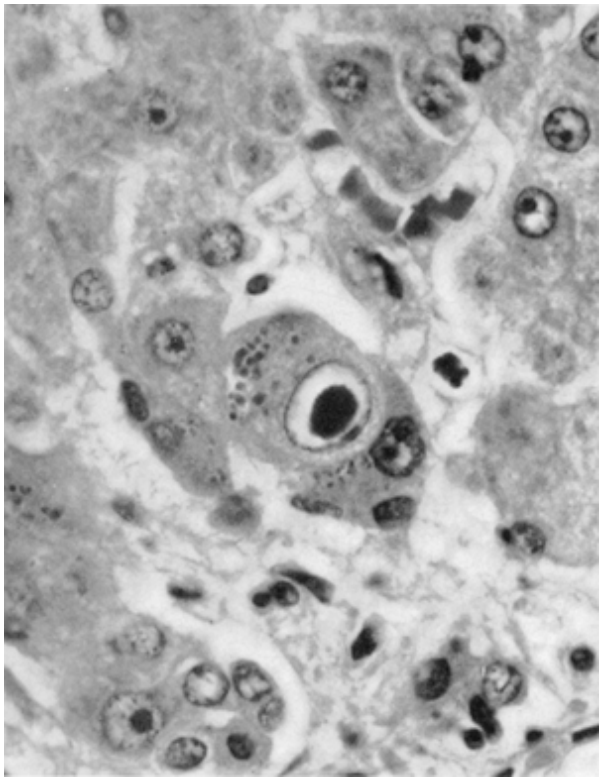
portal mononuclear cells. Apoptosis is present but ballooning is absent or minimal. Kupffer cells are often markedly hypertrophied and sometimes form tiny granulomatoid foci or, rarely, true granulomas. The hepatic sinusoids characteristically contain an increased number of lymphocytes, sometimes closely packed together in a "string of beads" pattern (Fig. 9.25). In CMV infection, cytomegaly and viral inclusions are never seen in immunocompetent patients, but in CMV infections of

the newborn, and of adults who are immunocompromised, characteristic intranuclear and cytoplasmic inclusions (Fig. 9.26) may be present in the bile duct and liver cells.



▪ **Figure 9.25** Infectious mononucleosis hepatitis. There is a prominent sinusoidal mononuclear cell infiltrate along with the hepatocellular injury of an acute hepatitis.

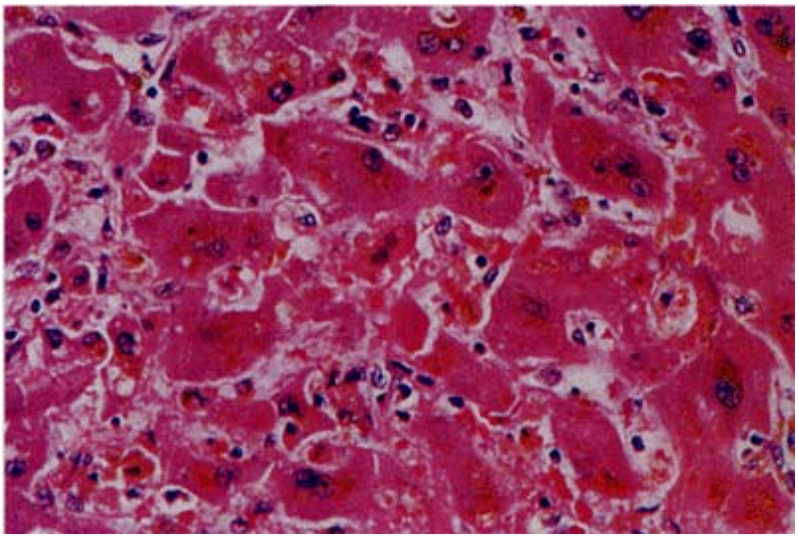
Cholestatic hepatitis, that is, combined hepatocellular and cholestatic injury, is an uncommon complication of most types of acute viral hepatitis, but it is frequent in hepatitis E infection (22). As a pattern of injury, it is more frequently seen in reactions to a number of drugs (23). Synonyms for cholestatic viral hepatitis include "cholangiolitic" or "pericholangitic" viral hepatitis, while drug-induced injury is termed *hepatocanalicular* or *mixed hepatocellular and cholestatic*. The clinical and laboratory findings tend to simulate those of obstructive biliary tract disease. The histopathology of cholestatic hepatitis includes hepatocellular and canalicular bile stasis, often with pseudogland formation, and variable degrees of parenchymal injury (Fig. 9.27). There may be periportal ductular proliferation with infiltration by neutrophils (acute cholangiolitis), as well as many neutrophils in the portal inflammatory infiltrate, but the acinar bile ducts are not involved.



• **Figure 9.26** Cytomegalovirus infection in an immunodeficient host. The large cell in the center of the field has a characteristic intranuclear inclusion and many small cytoplasmic inclusions.

Neonatal giant cell hepatitis typically has features of acute hepatitis with transformation of hepatocytes into multinucleate giant cells. Newborns with this disease typically present with jaundice, and the principal differential diagnosis is between this and extrahepatic biliary atresia (see Chapter 47). The term *idiopathic neonatal hepatitis* is used for most cases in which no cause is found, but the features seen in some neonates with α_1-antitrypsin deficiency may be identical to those seen in a number of other metabolic disorders and infections. The "idiopathic" cases are presumably secondary to undiagnosed viruses because most patients recover without sequellae. Histologically, all the features of acute hepatitis are present, along with significant bile stasis, but the most striking feature is giant cell transformation of hepatocytes (Fig. 9.28). The giant cells appear to result from the fusion of several liver cells to form a syncytium, and there may be up to several dozen nuclei in a single cell. The cholestasis may be quite prominent and there is often extramedullary hematopoiesis. Portal fibrosis and ductular proliferation, typical of biliary atresia, are not seen. Hepatocellular injury with giant

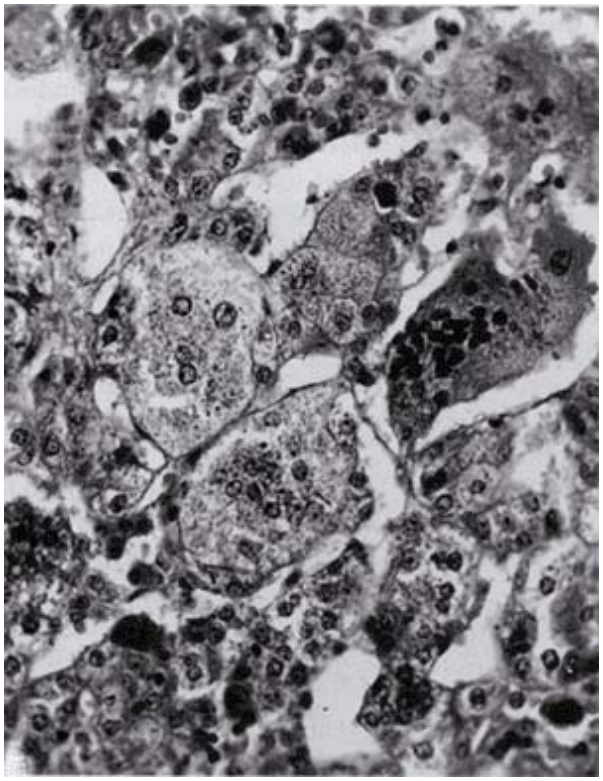
cell transformation also occurs in neonatal hemochromatosis, along with massive iron overload.



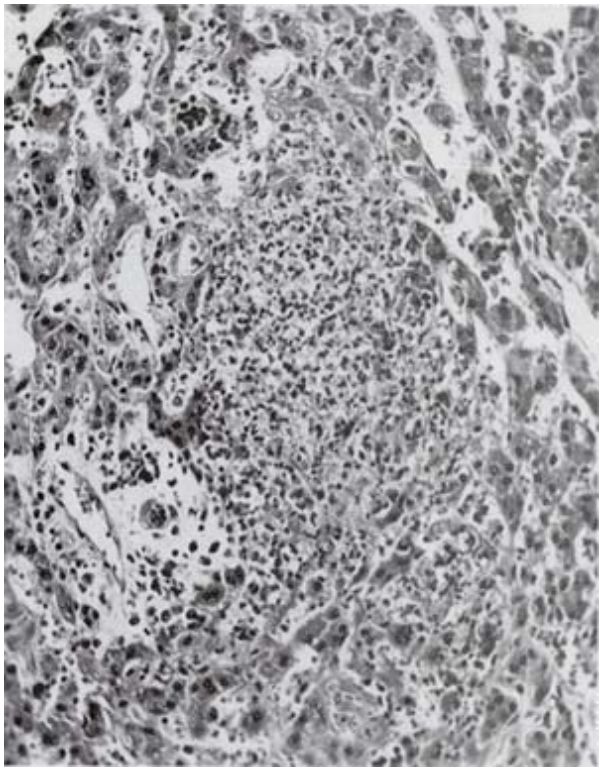
▪ **Figure 9.27** Acute cholestatic hepatitis (combined hepatocellular–cholestatic injury). There is prominent cholestasis, as well as hepatocellular injury, acidophilic bodies, liver cell dropout, and inflammation.

Acute injury with microabscess formation is typical of a number of bacterial infections complicated by sepsis or hematogenous dissemination. This includes diseases caused by both gram-positive and gram-negative organisms, such as listeriosis (Fig. 9.29), melioidosis, and typhoid fever, as well as disseminated mycotic infections such as those caused by *Cryptococcus, Candida*, or *Aspergillus* species. Organisms may or may not be

demonstrable in the lesions. In the immunocompromised host, CMV infection can also lead to this type of tissue response (with or without viral inclusions) in the liver. In all these diseases, lesions typically consist of varying-sized microabscesses, but they sometimes have a granulomatoid appearance. In CMV infection, the lesions are quite small, often only a single degenerating hepatocyte surrounded by neutrophils (sometimes containing an intranuclear inclusion), whereas bacterial and fungal infections may have grossly visible abscesses. In the later stages, lesions may have a purulent center and an organized granulomatous periphery, with variable fibrosis, especially in diseases such as meliodosis or typhoid.



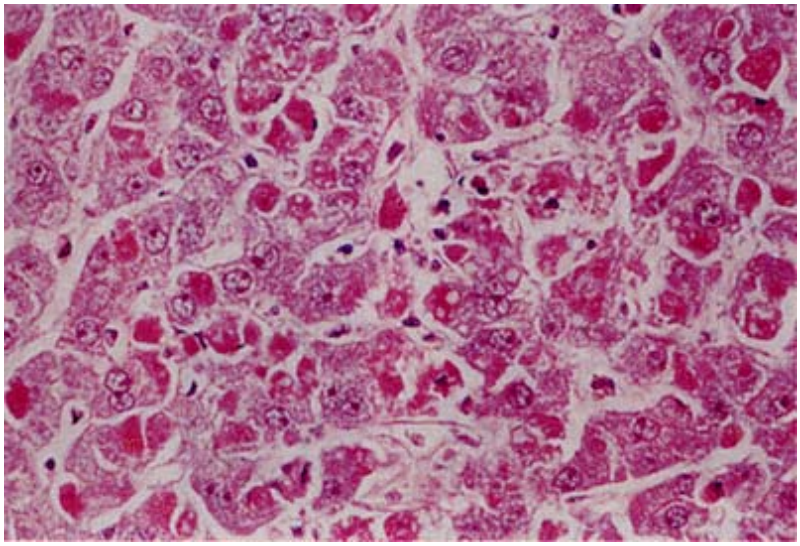
▪ **Figure 9.28** Idiopathic neonatal hepatitis with giant cell transformation. Note the enlarged hepatocytes containing numerous nuclei.



▪ **Figure 9.29** Listeriosis. A microabscess is present in the center of the field because of hematogenous dissemination of the infection.

Not all focal necrosis with a neutrophilic response or microabscess formation is infectious. Focal necrosis with an outpouring of neutrophils is also the characteristic reaction to degenerating liver cells harboring Mallory bodies in steatohepatitis, alcoholic or nonalcoholic. Perivenular focal necrosis with neutrophilic aggregation is an iatrogenic artifact often observed in open surgical biopsy specimens of the liver.

Acute injury with focal coagulative necrosis is seen in some viral infections in children, for example, coxsackie B4 and B9, but more importantly, this type of injury is typical of hepatic involvement in many types of viral hemorrhagic fevers, including yellow fever (Fig. 9.30), dengue, Lassa fever, and others (24). Haphazardly distributed single liver cells or clusters of liver cells are affected by coagulation necrosis, often with little or no inflammatory response. Viral inclusions are not present in any of these diseases.



▪ **Figure 9.30** Yellow fever. Many individual hepatocytes display coagulative necrosis in a haphazard distribution.

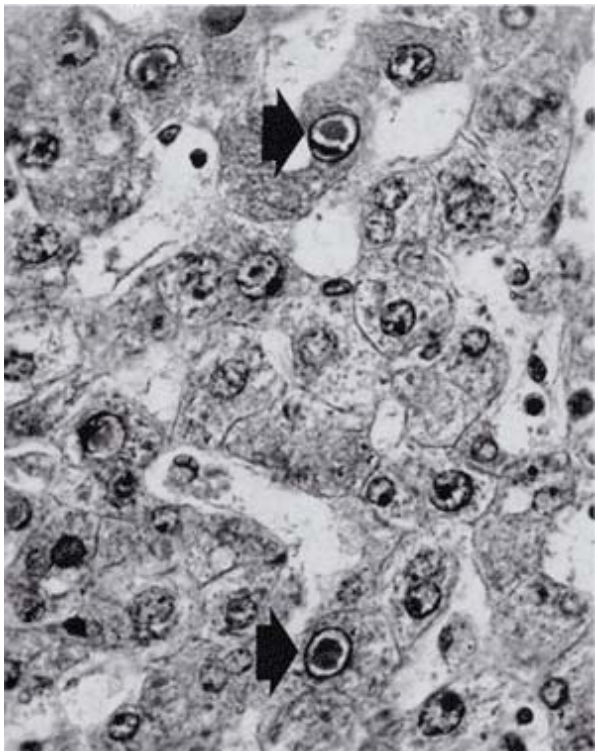
Acute injury with patchy or confluent coagulative necrosis is seen in disseminated herpes simplex hepatitis, whether occurring in neonates, children, or adults (25), both immunocompetent and immunocompromised. Similar findings are rarely seen in adenovirus hepatitis in immunocompromised patients (26). Viral inclusions are most easily identified in the relatively preserved hepatocytes at the margins of the necrotic foci. In herpes simplex, the classical Cowdry type A inclusions are eosinophilic, rounded or irregular, and surrounded by a clear halo with margination of the chromatin (Fig. 9.31). Adenovirus inclusions are more pleomorphic. Some resemble the Cowdry type A inclusions of herpes simplex but many are more basophilic and irregular in contour.

Acute injury with zonal submassive or massive coagulative necrosis is typical of toxic injury from a number of direct hepatotoxins, although it may also be produced by ischemic injury. Among toxins, acetaminophen overdose is by far the most frequent cause of this type of injury (27). The tissue maintains its acinar architecture, but hepatocytes in zone 3 are entirely necrotic (Fig. 9.32), with progressive involvement of zones 2 and 1 with increasing severity of injury. In the most severe cases, only a thin rim of viable hepatocytes surrounds each portal area.

Acute hepatitis with submassive necrosis and stromal collapse can be seen with severe hepatitis of any cause. This pattern of injury is more frequently encountered in biopsy and with surgical material from patients having drug-induced liver disease than in viral hepatitis, partly because patients with acute viral hepatitis are rarely subjected to

biopsy and because drug-induced injury tends, on average, to be more severe than viral

hepatitis, and also because many drugs are preferentially metabolized by hepatocytes of zone 3, thereby having the greatest toxic effects in this part of the acinus. There are patients who have not been exposed to a drug or chemical but develop fulminant liver failure with submassive or massive hepatic necrosis and in whom no evidence for any of the known hepatitis viruses can be found serologically or by PCR for viral DNA or ribonucleic acid (RNA) (27,28). These may be caused by some as yet undiscovered viral agent, but for now they remain enigmatic and unclassified. Finally, autoimmune hepatitis, although considered a chronic disease, often has an acute clinical presentation, and biopsies from the patients with this type of hepatitis may also show an appearance of acute hepatitis with submassive necrosis (29).



▪ **Figure 9.31** Herpes simplex hepatitis. Several of the nuclei have eosinophilic Cowdry type A inclusions surrounded by a clear halo with margination of the chromatin (*arrows*).

Submassive necrosis of any cause is due to the simultaneous death of the hepatocytes of an entire zone or more of the hepatic acini, thereby producing confluent necrosis, lysis of the necrotic tissue, and collapse of the supporting stroma. The necrosis usually involves zone 3 and, less often, zone 2 of the hepatic acini (Fig. 9.33), but viral hepatitis A and

injury due to some drugs and toxins are characterized by necrosis predominantly involving zone 1 (Fig. 9.34). In various planes of the section, acinar zone 3 necrosis may appear to be entirely around the terminal hepatic venule ("centrilobular"), may appear to extend between the terminal venules of adjacent acini, or may extend from the terminal venule to the edge of the portal area. Consequently, when necrosis affects zone 3, the collapsed reticulin framework may extend between adjacent vascular structures, making them appear linked together ("bridging necrosis"), and there may be linkage of portal areas with terminal hepatic venules ("portal–central bridging") or of two or more terminal hepatic venules ("central–central bridging"), both due to submassive zone 3 necrosis.

Acute hepatitis with massive necrosis, the most extreme form of acute hepatocellular injury, may be caused by viral hepatitis or drug-induced liver disease. There is virtually complete loss of hepatocytes (Fig. 9.35), but occasionally, the haphazardly distributed cells can survive, as can a cuff of cells around portal areas. The reticulin framework is usually intact but frequently collapsed because of loss of liver cells, with resultant approximation of portal areas. Variable numbers of inflammatory cells are present in the areas of collapse. These include lymphocytes and plasma cells, as well as a lesser number of eosinophils and neutrophils. Central vein endophlebitis may be present. The collapsed parenchyma contains numerous hypertrophied Kupffer cells with cytoplasm packed with lipofuscin. Zone 1 of the hepatic acinus typically shows proliferation of the putative hepatic stem cells (Fig. 9.36), forming ductules and ductular hepatocytes (30).

Chronic Necroinflammatory Injury (Chronic Hepatitis)

Chronic necroinflammatory disease refers to a morphologic pattern that is seen most often not only in chronic viral hepatitis but also in autoimmune hepatitis, occasional drug reactions, and, in rare instances, some metabolic diseases. As in acute necroinflammatory disease, there is hepatocellular injury and inflammation, but in the chronic diseases, the brunt of the injury tends to be portal and periportal rather than panacinar, and the injury is accompanied by fibrosis that can progress to cirrhosis. Chronic hepatitis, regardless of cause, is characterized by several pathologic changes that are present to a variable extent in each case. These include portal inflammation and sometimes lesions of bile ducts within the portal spaces; periportal injury and inflammation; several forms of degeneration and death by apoptosis of intra-acinar hepatocytes with an associated inflammatory response; and fibrosis that may involve only the portal and periportal areas or that may form septa.

Morphology of chronic hepatitis

Portal inflammation—in all forms of chronic hepatitis the portal areas are variably infiltrated by lymphocytes and plasma cells. Lymphoid aggregates or follicles with

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germinal centers may be present and are now considered typical, although not pathognomonic, of chronic hepatitis C (Fig. 9.37). Immunohistochemical studies have shown that even when germinal centers are not apparent by light microscopy, these are true functional lymphoid follicles (31). The germinal centers contain activated B cells surrounded by a follicular dendritic cell network and a mantle zone of B cells, which, in turn, is surrounded by a T-cell zone. Patients with autoimmune hepatitis will often

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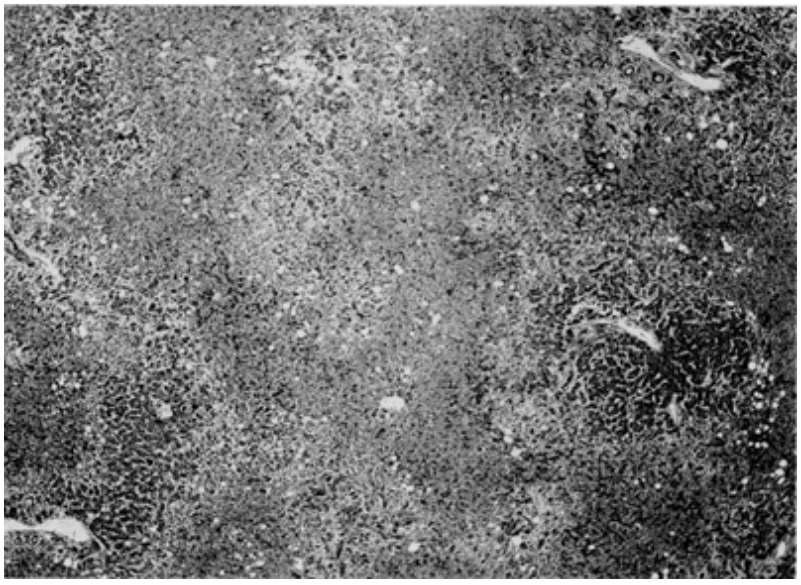
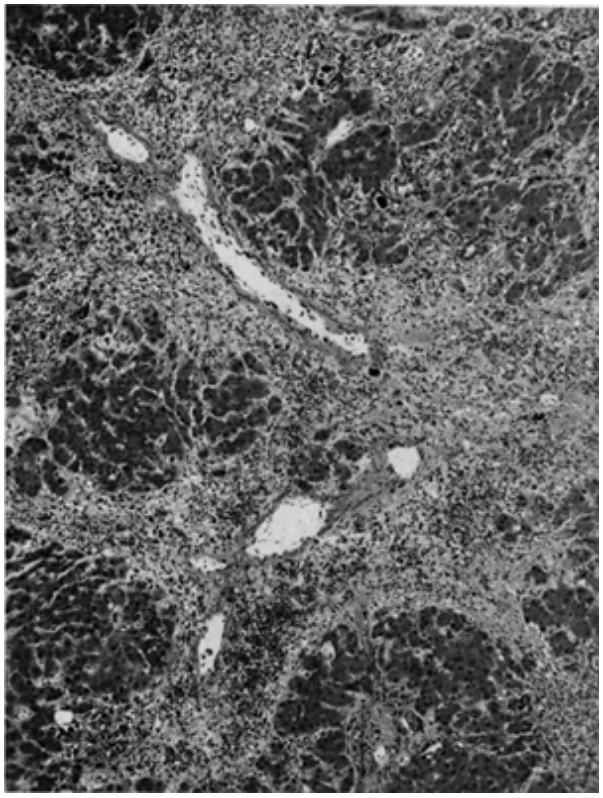
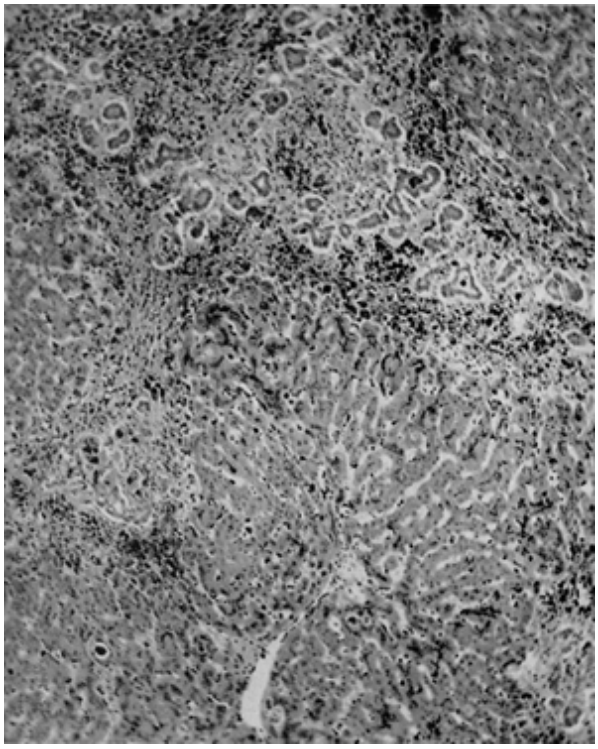
have large numbers or plasma cells in the portal inflammatory infiltrate (Fig. 9.38). Biopsy specimens from patients who are affected by chronic hepatitis through intravenous drug abuse may have birefringent talc crystals in portal macrophages (Fig. 9.14) (10).



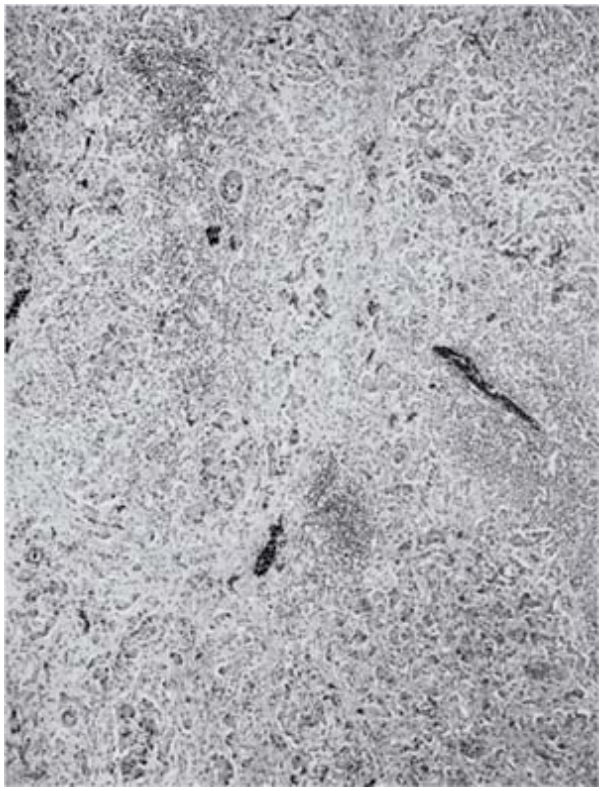
▪ **Figure 9.32** Submassive zonal coagulative necrosis in a fatal case of acetaminophen overdose. The necrotic hepatocytes of zones 2 and 3 are present in the section, while some of the zone 1 (periportal) cells survive.



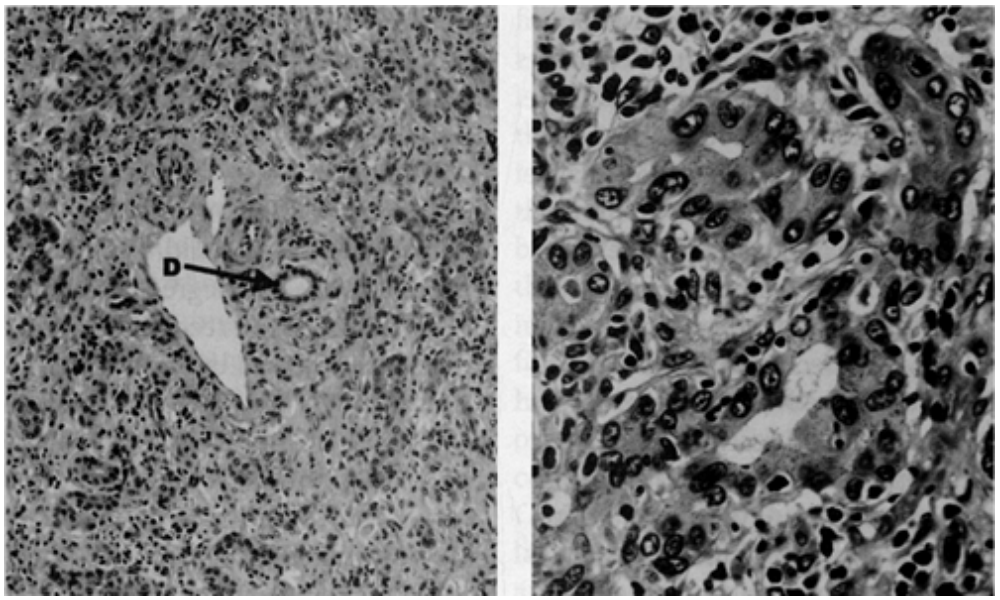
▪ **Figure 9.33** Acute submassive necrosis of acinar zones 3 and 2. Surviving liver cells are predominantly periportal in zone 1 of the acini. In contrast to the coagulative necrosis shown in Figure 9.32, there is a collapse of the stroma where the hepatocytes have been lost.



▪ **Figure 9.34** Acute submassive necrosis of zone 1. With the Prussian blue stain, hemosiderin-laden macrophages (dark-staining) are shown to outline the areas of periportal necrosis and liver cell loss.

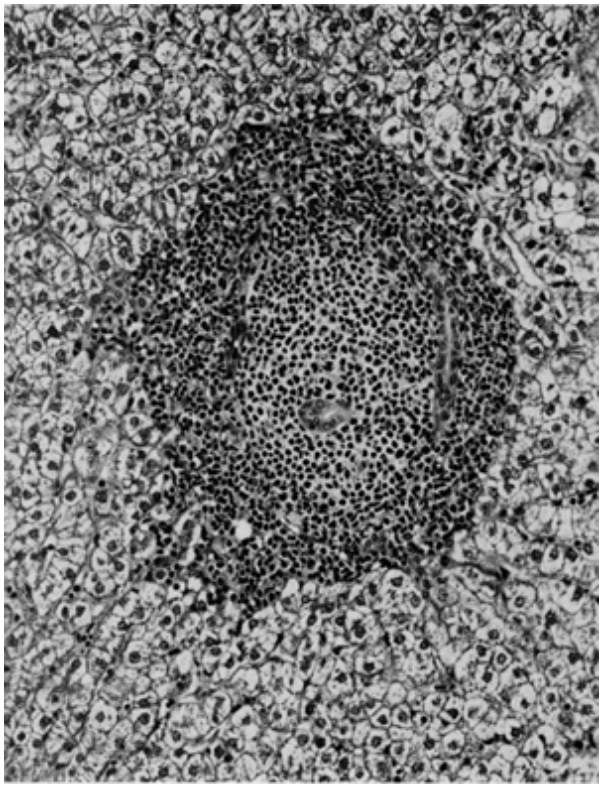


▪ **Figure 9.35** Acute hepatitis with massive hepatic necrosis. There is loss of all hepatocytes with proliferation of ductules in the collapsed hepatic stroma.

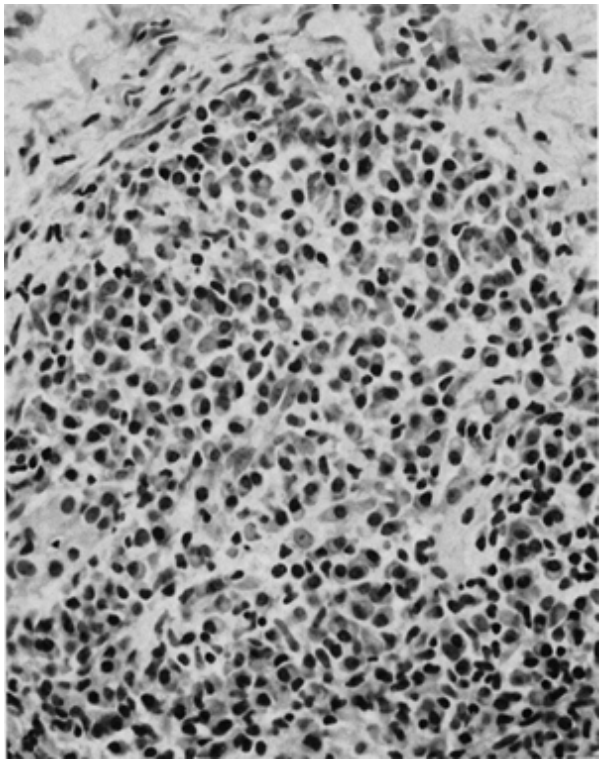


▪ **Figure 9.36** Ductular proliferation in massive hepatic necrosis.

The putative stem cells of the liver have proliferated, forming ductules and differentiating into hepatocytes in a vain attempt to repopulate the liver. The *left panel* has a portal area with an acinar bile duct (*D*) surrounded by collapsed stroma that contains proliferating ductules and inflammatory cells. The *right panel* shows ductules at high magnification. Some of the ductular cells have granular, eosinophilic cytoplasm, indicating differentiation into hepatocytes.



▪ **Figure 9.37** Chronic portal inflammation with a lymphoid aggregate that has a germinal center in a patient with chronic hepatitis C.



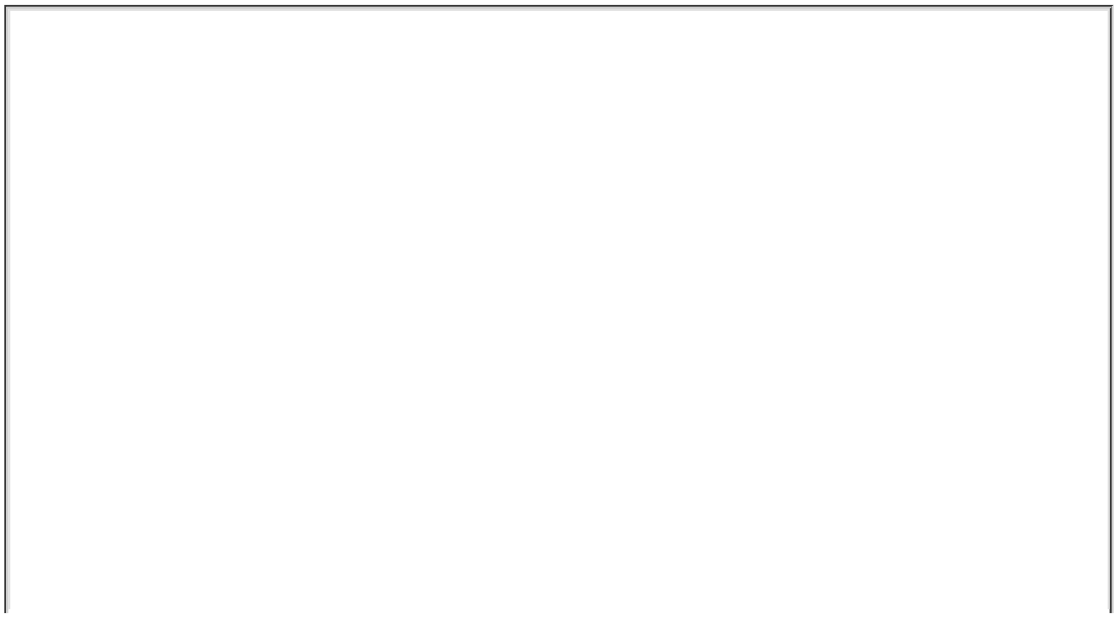
▪ **Figure 9.38** Autoimmune hepatitis with large number of portal plasma cells, recognizable by their eccentric nuclei and clear perinuclear Golgi zone.

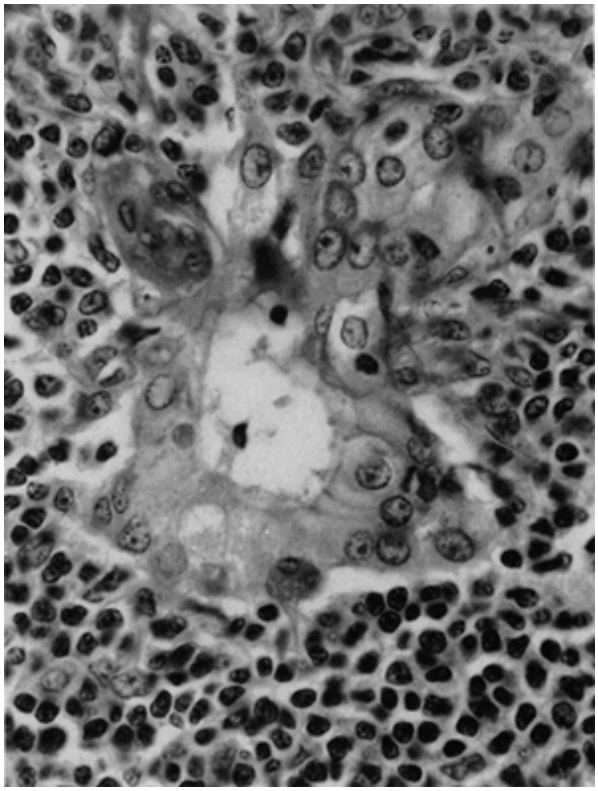
Hepatitis-associated bile duct lesions were first described in chronic hepatitis (32), but lesions may also be found in biopsy specimens of acute hepatitis. The lesion is characterized by swelling, vacuolization, nuclear irregularity, and sometimes, pseudostratification of the biliary epithelial cells (Fig. 9.39). The basement membrane may appear to be ruptured, and lymphocytes, occasional plasma cells, and sometimes, neutrophils infiltrate the duct. The lesion is reminiscent of and sometimes indistinguishable from the "florid duct lesions" of PBC. However, in contrast to the lesions of PBC, the ducts are not destroyed, and so portal areas without ducts are seldom seen and features of chronic cholestasis do not develop. Serial section reconstruction studies (33) have demonstrated that the most frequently observed lesions are actually blind diverticula arising from injured ducts rather than the ducts themselves. The ductal lesions have been seen in all forms of hepatitis, but most commonly in hepatitis C (34).

Interface hepatitis is now the preferred term for the lesion formerly known as *piecemeal necrosis* (35). The original term was defined by an international group as "the destruction of liver cells at an interface between parenchyma and connective tissue, together with a

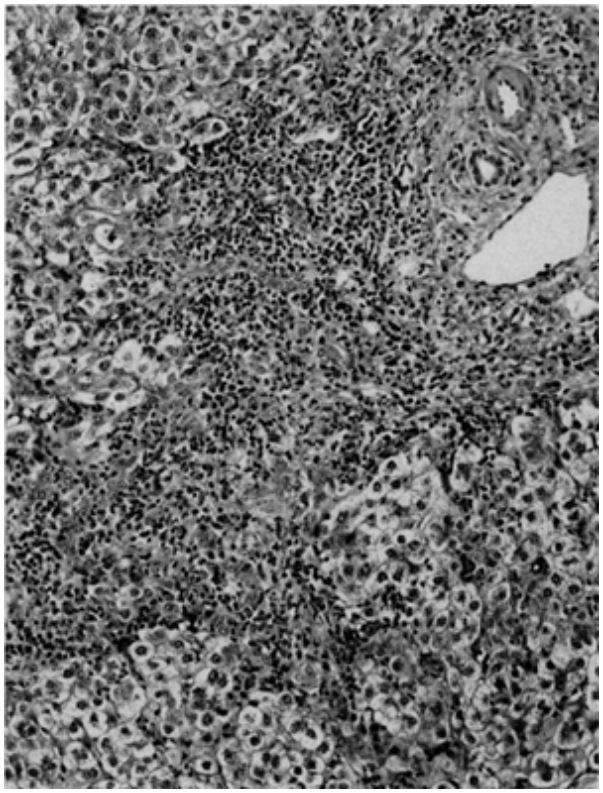
predominantly lymphocytic or plasma cell infiltrate" (36). It is now apparent that the destruction of liver cells is primarily through apoptosis, and because the dead hepatocytes quickly disappear from the tissue, it is the location of the inflammatory component that permits recognition of the lesion, making *interface hepatitis* the more accurate term. *Interface hepatitis* has long been considered to be a key lesion in the progression and pathogenesis of chronic hepatitis, and the degree of periportal injury (mild, moderate, or marked) is still used to grade the degree of activity. Interface hepatitis can be most easily recognized as irregularity of the limiting plate, caused by extension of the portal inflammation through the plate into the periportal parenchyma (Fig. 9.40). The limiting plate becomes irregular and may disappear as the portal area expands. Inflammatory cells surround and invade injured hepatocytes ("emperipolesis"). There may be evidence of hepatocellular degeneration and death, characterized by either acidophilic or ballooning degeneration. As in acute hepatitis, cell death occurs principally by the process of apoptosis, resulting in the formation of apoptotic or acidophilic bodies, which rapidly disappear from the liver

plates or sinusoids. As chronic hepatitis progresses, there is continuous erosion of the hepatic parenchyma, with closer and closer approximation of expanded portal areas, and small groups of hepatocytes ("hepatocytic islets") become trapped in expanded portal zones. The necroinflammatory changes are gradually succeeded by fibrosis, often best appreciated with a Masson or other collagen stain. Delicate collagen fibers laid down in areas of periportal liver cell loss eventually condense into scars. Interface hepatitis may not involve all the portal areas equally in a given biopsy specimen. It may affect either a segment or the entire perimeter of a portal area. Furthermore, even after cirrhosis has developed, interface hepatitis can continue unabated along the fibrous septa, causing further loss of parenchyma and, eventually, clinical decompensation of the cirrhosis.





▪ **Figure 9.39** Hepatitis-associated bile duct lesion with marked epithelial injury and infiltration by chronic inflammatory cells, similar to the florid duct lesions of primary biliary cirrhosis.



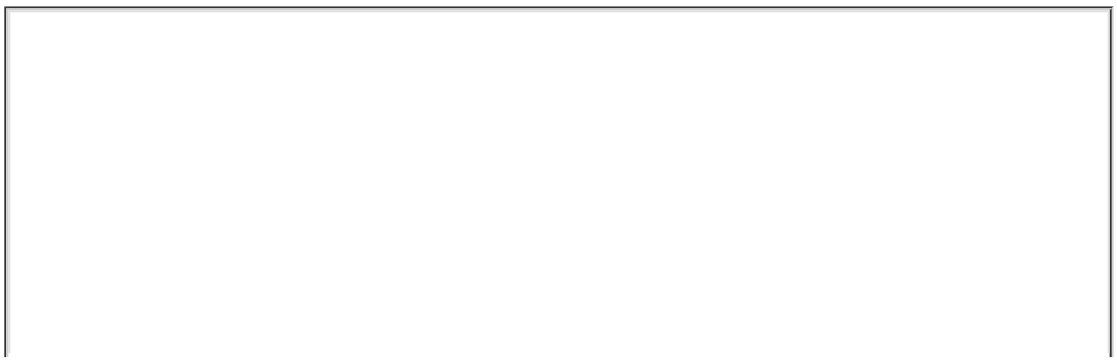
▪ **Figure 9.40** Interface hepatitis ("piecemeal necrosis") can be most easily recognized as irregularity of the limiting plate, caused by extension of portal inflammation through the plate into the periportal parenchyma.

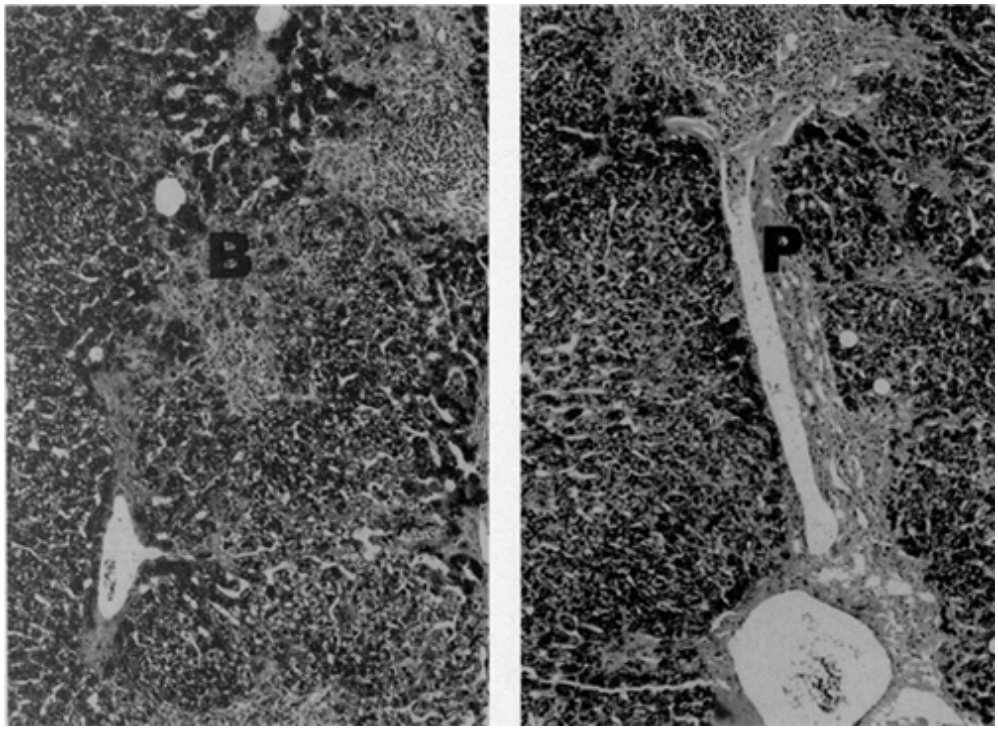
Parenchymal injury, causing intra-acinar necroinflammatory changes, is present to some degree in most biopsy specimens from patients with any type of chronic hepatitis. This is typically multifocal ("spotty") in distribution and consists mainly of apoptosis, as in acute hepatitis. Scattered apoptotic bodies of varied size are observed, as well as focal aggregates of lymphocytes, plasma cells, and hypertrophied Kupffer cells that have scavenged the apoptotic bodies and other debris, producing lesions traditionally called *focal or spotty necrosis*. More severe intra-acinar injury is generally seen when the biopsy is performed during an acute exacerbation of the chronic hepatitis, even if the patient is asymptomatic. Changes typical of acute hepatitis, superimposed on those of chronic hepatitis, may include an increase in the degree of spotty necrosis; ballooning degeneration, often most severe in zone 3, with dropout of hepatocytes and central–central or central–portal bridging necrosis, especially in autoimmune hepatitis; variable cholestasis, often with associated periportal ductular proliferation and infiltration of the ductules by neutrophils; or (in extreme cases) multiacinar necrosis with stromal collapse. There is

simultaneous regeneration of hepatocytes as cells are lost through apoptosis. This is typically seen in the form of two-cell thick plates and an increased number of bi- and trinucleated hepatocytes, but mitotic figures may occasionally be present. There may be some degree of steatosis, generally macrovesicular and of mild to moderate severity, most often in hepatitis C but also in chronic hepatitis of other causes.

Fibrosis is an almost invariable part of chronic hepatitis, although the degree of fibrous tissue deposition is quite variable from patient to patient. Fibrosis is the progressive component of the disease because it is the fibrous scarring that leads to architectural distortion and cirrhosis. It is thought that at least two pathways may lead to the fibrosis of chronic hepatitis. Probably most important in chronic viral hepatitis is the collagen deposition that accompanies the periportal injury of interface hepatitis, causing fibrous expansion of the portal tracts. As the disease progresses, portal–portal fibrous bridges are formed, filling zone 1 between adjacent acini. There may also be formation of "central–portal" and sometimes central–central fibrous bridges, which can develop from superimposed episodes of necrosis involving zone 3. In addition, it is likely that broad areas of fibrosis can result from the healing of bouts of multiacinar necrosis or from ischemic injury due to vascular damage secondary to the inflammation (see Chapter 7). In evaluating needle biopsies, it is important to distinguish tangential cuts through enlarged portal areas, which contain preexisting bile ducts and portal vessels, from true bridging fibrosis, which forms septa through parenchyma that had no preexisting fibrous tissue (Fig. 9.41). The scars of bridging fibrosis contain elastic fibers in addition to collagen. Like scars in any tissue, these tend to contract. Contraction of the fibrous septa in concert with nodular regeneration of the surviving parenchyma produces architectural distortion, and when complete nodules have formed, surrounded by fibrous septa, the result is the development of cirrhosis. Before the architecture is entirely obliterated, parts of the tissue are nodular while whereas adjacent areas maintain an acinar structure, a

state that can be regarded as an "incomplete" cirrhosis. When necroinflammatory changes continue along the septa and within the nodules, this may be considered active cirrhosis, or a term such as *chronic hepatitis with cirrhosis* may be used.





▪ **Figure 9.41** Bridging fibrosis (*B*) is scar tissue that forms across an area of parenchyma that had no preexisting fibrous tissue. It must be distinguished from tangential cuts through fibrotic preexisting portal areas (*P*), which contain bile ducts and arteries.

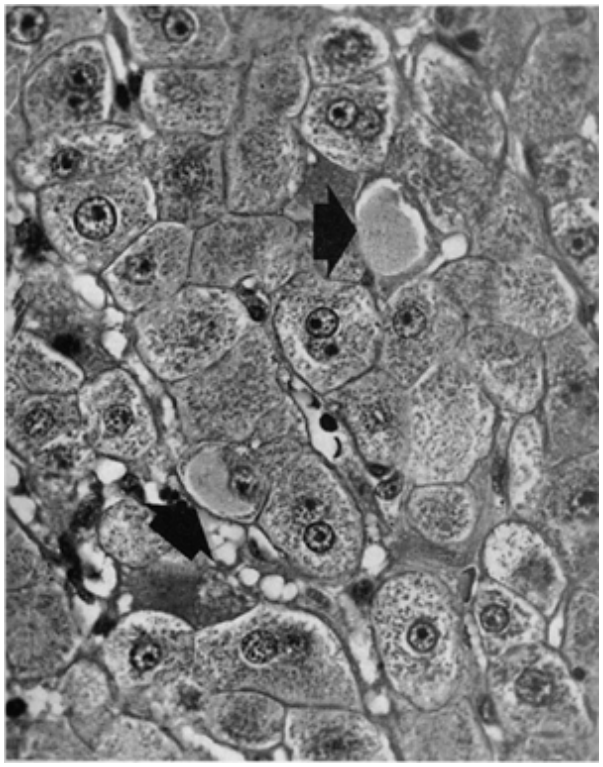
There are several known causes of chronic hepatitis, and although the histopathologic features are similar, there are some noteworthy features that are more characteristic of one type than another. Parenterally transmitted forms of viral hepatitis, which account for at least 90% of cases, are discussed in detail in Chapters 29 and 30. Approximately 5% to 10% of chronic hepatitis is autoimmune (see Chapter 31). Drug-induced liver disease is a rare but well-documented cause of chronic hepatitis (23), and if the other known causes are excluded, this should always be considered and evaluated clinically with a complete drug history. Metabolic diseases, such as Wilson disease, α_1-antitrypsin deficiency, and hemochromatosis are sometimes listed in textbooks and reviews as causes of chronic hepatitis, but because these are usually readily distinguished from chronic hepatitis by liver biopsy and laboratory tests, they are considered separately in this chapter.

Hepatitis B can be diagnosed histologically and distinguished from other causes of chronic hepatitis by demonstration of the virus in tissue. Hepatitis B surface antigen (HBsAg) can be demonstrated by histochemical stains (orcein or Victoria blue, Fig. 9.8) or the more sensitive immunostains (Fig. 9.13) in 80% or more cases of chronic

hepatitis B. Cells containing large quantities of HBsAg have cytoplasm with a uniform finely granular appearance, the so-called ground-glass cells (Fig. 9.42), which are scattered randomly through the liver, often occurring in clusters. The number of ground-glass cells tends to be inversely related to the activity of the hepatitis. Most cells are found in livers with the least active disease, whereas livers with the most activity tend to have the fewest ground-glass cells. In acute hepatitis B, the immune response eliminates antigen-containing cells and immunostains are entirely negative. Conversely, the presence of stainable surface antigen proves a chronic rather than an acute infection, even when there is severe hepatocellular injury. Hepatitis B core antigen (HBcAg) can also usually be immunohistochemically demonstrated in nuclei, and sometimes cytoplasm when there is chronic disease. The presence of core antigen reflects active viral replication, and so the amount of core tends to be directly proportional to the activity of the hepatitis. Patients with recent acute exacerbations will have the most core and will often have HBcAg in hepatocyte cytoplasm and in numerous nuclei. Strains of virus with precore mutations associated with increased disease severity have also been found to have increased cytoplasmic core antigen (37).

Hepatitis C virus cannot currently be reliably demonstrated in routinely processed liver biopsies. There

are, however, histologic features that are characteristic, although not pathognomonic, of chronic hepatitis C (34), and the finding of these features should prompt a serologic evaluation if not already done. Chronic hepatitis C tends to have more intense chronic portal inflammation than other types of chronic hepatitis, often with lymphoid aggregates and sometimes follicles with germinal centers (Fig. 9.37). There is also a greater tendency toward hepatocellular fat accumulation than in other types. Approximately 50% of biopsy specimens have some fat, and in approximately 10% this may be considerable. Patients infected with genotype 3 tend to have even more fat, and it is suggested that this may be due to a cytopathic effect (38). Hepatitis-associated bile duct lesions (see preceding text) may be found in acute or chronic hepatitis of any cause, but they are most frequent in hepatitis C. Severe degrees of bile duct injury can be seen in approximately 10% to 15% of biopsy specimens from patients with chronic hepatitis C (Fig. 9.39). Lesser degrees of duct irregularity and lymphocytic infiltration can be found more often.



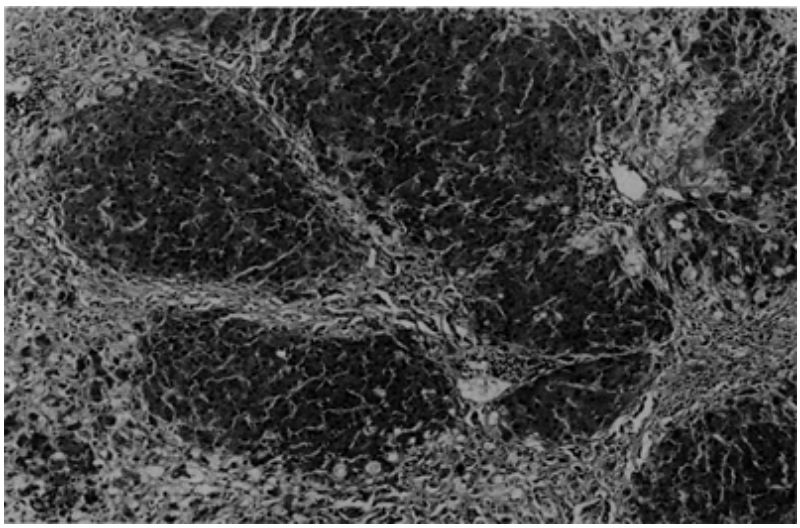
▪ **Figure 9.42** Ground-glass cells (*arrows*) in chronic hepatitis B. The cytoplasm of liver cells containing large quantities of hepatitis B surface antigen (HBsAg) has a uniform finely granular appearance.

Hepatitis D can only infect individuals who are also infected with HBV, which serves as an obligatory helper. Simultaneous coinfection with HBV and hepatitis delta virus (HDV) tends to cause more severe disease than HBV alone, with a higher likelihood of fulminant hepatitis. HDV superinfection of a person with previous chronic HBV infection often causes an acute exacerbation of the underlying chronic hepatitis or deterioration in the clinical status of a previously stable patient, or it may cause fulminant liver failure. Morphologically, HDV superimposed on HBV tends to produce more severe disease than hepatitis B alone, but there are no features that specifically implicate hepatitis D (39). The only way to histologically prove presence of the virus is to demonstrate delta antigen in the hepatocyte nuclei by immunohistochemical staining (although commercial antibodies are not widely available) or to detect antibodies to delta antigen in the blood.

Autoimmune hepatitis tends to be a very severe chronic hepatitis, often with multiacinar collapse and/or cirrhosis at the time of presentation. Numerous plasma cells are often seen in the portal inflammatory infiltrate (Fig. 9.38). About one third of cases have an acute onset, and,

typically, there is severe acute hepatitis-like hepatocellular injury, often with diffuse ballooning of hepatocytes, regeneration with hepatocyte rosette formation, and sometimes, confluent zone 3 hepatocellular necrosis (29). Extensive giant cell transformation (40) is seen in some cases, which have been called *postinfantile giant cell hepatitis* or *syncytial giant cell hepatitis*.

Recurrent chronic hepatitis after liver transplantation may resemble the original disease or may have atypical features. Hepatitis B, before antiviral therapy became established as standard post-transplantation therapy, virtually always recurred and in many cases caused a severe, rapidly progressive disease in immunosuppressed patients. The term *fibrosing cholestatic hepatitis* was proposed for this form of hepatitis B (41). Liver biopsy specimens show numerous ground-glass hepatocytes with massive amounts of intracellular HBsAg and HBcAg, and it is thought that this represents a cytopathic form of viral infection, in contrast to the usual chronic hepatitis B. As the disease progresses, there is portal and diffuse pericellular fibrosis, hepatocyte dropout, and in the late stages, nodular regeneration, producing cirrhosis (Fig. 9.43). Cholestasis in the tissue may be severe, but the patients typically have elevated serum bilirubin concentration even when bile pigment is histologically inapparent, hence the "cholestatic" part of the name. Hepatitis C also recurs, but most patients have histologic features of typical chronic hepatitis. Occasionally, however, patients display features of fibrosing cholestatic hepatitis, except for ground-glass cells (42). It is not clear whether the pathogenesis is the same as that for hepatitis B. Both hepatitis B and C occasionally produce the pattern of fibrosing cholestatic hepatitis in patients who have not undergone liver transplantations but are immunosuppressed or immunodeficient for other reasons (43).



▪ **Figure 9.43** Recurrent hepatitis B with fibrosing cholestatic

hepatitis progressing to cirrhosis 1 year after liver transplantation. The surviving hepatocytes have abundant cytoplasmic hepatitis B surface antigen (HBsAg), producing a ground-glass appearance, and there is canalicular bile stasis.

Grading and staging of chronic hepatitis

The stage of any disease is a measure of how far it has progressed in its natural history, with the end stage resulting in death of the patient or failure of the organ. The grade of the disease is meant to reflect how quickly the disease is progressing to the end stage. In chronic hepatitis the end stage is cirrhosis with clinical decompensation, whereas earlier stages have lesser degrees of fibrosis or cirrhosis. The grade is considered to be the degree of inflammation and hepatocellular injury, which is thought to lead to fibrosis.

The old terminology that classified chronic hepatitis as "chronic persistent hepatitis," implying a benign, nonprogressive disease, or "chronic active hepatitis," implying a disease with a high likelihood of progression to cirrhosis, was a form of grading, but advances in understanding the causes and elucidating the natural history of the diseases that produce this type of liver injury has rendered this terminology obsolete.

There are several methods, currently in use, of expressing the grade and stage of chronic hepatitis. These can be grouped into those that are simple verbal descriptions, those that are relatively simple numeric grades and stages that correspond to the verbal descriptions, and those that use more complicated numeric scoring of histologic features to generate numbers that correspond to the grade and stage. Each method has advantages and disadvantages, and the system used should be appropriate to its suitability for the task at hand. In general, more complex systems have the capability to provide more information than simple ones but are less reproducible.

For routine diagnosis and patient management a simple system of grading and staging is preferred, and we recommend the guidelines that were proposed by a panel of experts convened by the International Association for the Study of the Liver (IASL) in 1994 (44). Grading of chronic hepatitis is accomplished by deciding whether the degree of activity is mild, moderate, or marked. Although this seems rather simple, it is essentially subjective and not highly reproducible between pathologists or even by the same pathologist (45). The principal features used to determine grade are the degree of periportal interface hepatitis ("piecemeal necrosis") and spotty parenchymal injury (Table

9.1). We consider interface hepatitis to be mild when one must search for a foci in the biopsy specimen; moderate when most portal areas have some interface hepatitis, but it extends around less than 50% of the circumference in the majority; and marked when most portal areas have interface hepatitis extending around more than 50% of the circumference. Parenchymal injury is most easily graded using the 10× (medium power) objective of the microscope with the usual 10× ocular lens. At this magnification it is possible to detect acidophilic bodies, ballooned hepatocytes, and clusters of inflammatory cells at sites of focal necrosis, and it is relatively easy to estimate the amount of injury and form an overall impression of the degree of injury. We consider parenchymal injury to be mild when fewer than five injured cells or clusters

of inflammatory cells are seen per 10× field, moderate when there are 5 to 20, and marked when there are more than 20 per 10× field. Portal inflammation can also be considered, but this is more a sign of chronicity than of activity. In grading the overall activity, the chronic hepatitis can be considered as being mild when both interface hepatitis and parenchymal injury are mild or absent, moderate if both interface hepatitis and parenchymal necrosis are moderate or if one is moderate but the other is mild, and marked if interface hepatitis and/or parenchymal injury is marked.

Table 9.1. Grading Activity in Chronic Hepatitis

Grade	Interface hepatitis	Parenchymal injury[a]	Activity
Mild	Found only after diligent search	<5 per 10× field	*Both* interface hepatitis and parenchymal injury are *mild* or less
Moderate	Most portal areas have at least some, but most have <50% of circumference	5–20 per 10× field	*Either* interface hepatitis or parenchymal injury is *moderate*
Marked	>50% of	>20 per 10×	*Either*

	circumference of most portal areas	field	interface hepatitis or parenchymal injury is *marked*

[a]Apoptotic bodies, ballooned cells, inflammatory cell aggregates.

Staging of chronic hepatitis requires assessment of the degree of fibrosis, which requires a Masson trichrome stain for proper evaluation. There is progression in the stage of disease as fibrosis advances from none to fibrous portal expansion, bridging fibrosis, incomplete cirrhosis, and finally to established cirrhosis.

Following the recommendations of the IASL panel (44), the diagnostic line of the pathology report should indicate the cause of the chronic hepatitis and, if known, the grade and the stage. Therefore, the report may read "chronic hepatitis C with mild activity and portal fibrosis"; or "chronic hepatitis B with moderate activity and extensive bridging fibrosis"; or "chronic autoimmune hepatitis with marked activity and established cirrhosis." For those who prefer numbers to words, there are simple numeric scores that generally correspond to verbal diagnoses (Table 9.2), including the Batts-Ludwig (46) and Metavir (47) systems. We prefer the verbal diagnoses because these avoid the false sense of quantification that numbers tend to engender.

Complex numeric systems have also been proposed for grading and staging chronic hepatitis, including the Knodell Histology Activity Index (48), commonly called the *Knodell score*, and its modified form, known as the *Ishak score* (35). For grading, these systems assign numbers to the severity of the necroinflammatory features (e.g., interface hepatitis, confluent necrosis, parenchymal injury, and portal inflammation) and add these numbers to arrive at a grade that can range from 0 to 18. The stage, ranging from 0 to 4, may or may not be added into the Knodell score; in the Ishak score, the stage, ranging from 0 to 6, is reported separately. Numeric scores generated by these systems are useful for investigational studies that involve large numbers of patients requiring statistical analysis. These scores are a good way of showing differences in histologic response between cohorts of patients receiving different forms of therapy, and they have been used successfully in many large clinical trials. However, studies have

shown that there is fairly poor reproducibility for these scores when applied to *individual* liver biopsies, both between different pathologists and by the same pathologist at different times (45,49). When dealing with an individual liver biopsy, the pathologist should avoid complex numeric scores and concentrate on a meaningful verbal report.

Follow-up biopsies from patients who are being treated for chronic hepatitis should be evaluated in the context of their previous biopsies. The biopsy is performed in this clinical setting to see whether the activity of the patient's liver disease has improved or whether there has been progression of fibrosis. The only meaningful evaluation is one that compares the initial biopsy with the follow-up biopsy. It is essential that this be done by a single pathologist (although the clinician may wish to look on) comparing both biopsies together. A comparison of pathology reports

or numeric scores generated at different times can only lead to confusion and incorrect conclusions about the course of the patient's disease.

Table 9.2. Simple Numeric Grading and Staging SystemsT hat Correspond to Verbal Diagnoses

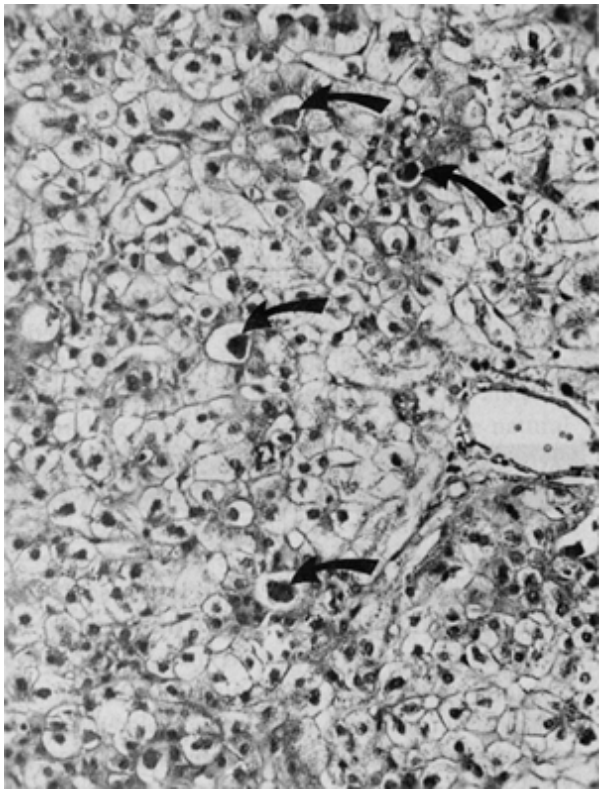
Grade	Metavir (47)	Batts-Ludwig (46)
Chronic hepatitis, minimal	A1	Grade 1
Chronic hepatitis, mild	A1	Grade 2
Chronic hepatitis, moderate	A2	Grade 3
Chronic hepatitis, marked	A3	Grade 4
Stage		
No fibrosis	F0	Stage 0
Portal fibrosis	F1	Stage 1

Few bridges	F2	Stage 2
Many bridges	F3	Stage 3
Cirrhosis	F4	Stage 4

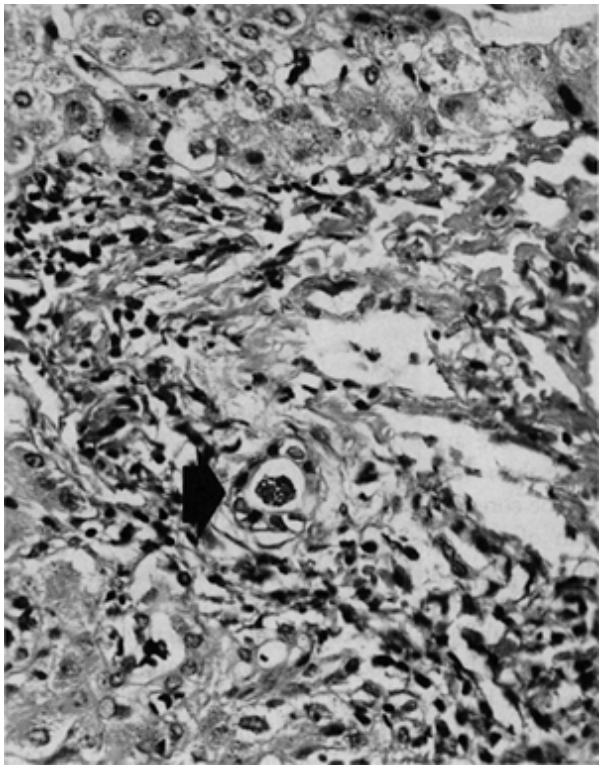
Acute Cholestasis

Cholestasis can be defined as the arrested flow of bile. Morphologically, when there is an acute impediment to bile flow, no matter the cause, bile pigment can usually be found in hepatocytes, canaliculi (Fig. 9.44), and sometimes Kupffer cells, predominantly in acinar zone 3 ("centrilobular"), except when hyperbilirubinemia is minimal. When jaundice is severe, especially in mechanical biliary obstruction, bile pigment may be seen in the lumina of ductules or acinar bile ducts (Fig. 9.45). Bile may be confused with other pigments, particularly hemosiderin and lipofuscin. It is typically a darker brown than the pale yellow lipofuscin. Lipofuscin is more granular and is more likely to be found near the cytoplasmic membrane. Hemosiderin is dark brown, glassy, and usually more refractile than bile or lipofuscin. The Prussian blue stain for iron is useful for identifying bile and so is Hall's stain for bilirubin.

Many disorders, including alcoholic liver disease, drug-induced hepatotoxicity, viral hepatitis, and various developmental and metabolic diseases, can be associated with intrahepatic cholestasis. In general these show features typical of the basic pattern of injury, and cholestasis is a minor component of the picture. This section deals with cholestasis as the predominant feature.



▪ **Figure 9.44** Multiple bile plugs (*arrows*) in zone 3 canaliculi in a patient with an acute onset of cholestatic jaundice.



▪ **Figure 9.45** Bile in an acinar bile duct (*arrow*) in extrahepatic biliary obstruction.

"Bland" cholestasis and cholestatic hepatitis

"Bland" cholestasis refers to an acute cholestatic injury unaccompanied by hepatocellular injury or bile duct injury. The cholestasis caused by some drugs, such as anabolic and contraceptive steroids, is typically "bland," unaccompanied by hepatocellular injury of more than a minimal degree (50). The differential diagnosis, however, includes mechanical biliary obstruction because in the early stages, portal area changes, as noted in the subsequent text, may not be present. In some patients, the cause of the "bland" cholestasis is never found. Benign recurrent intrahepatic cholestasis (BRIC) is a term used for a familial disorder caused by mutations of the *FIC1* gene that is also responsible for progressive familial intrahepatic cholestasis type 1 (PFIC-1) (51). BRIC is characterized by bouts of cholestasis that are self-limited and not followed by fibrosis or cirrhosis. Biopsy specimens show moderate to marked cholestasis and little or no hepatocellular injury. Similar findings

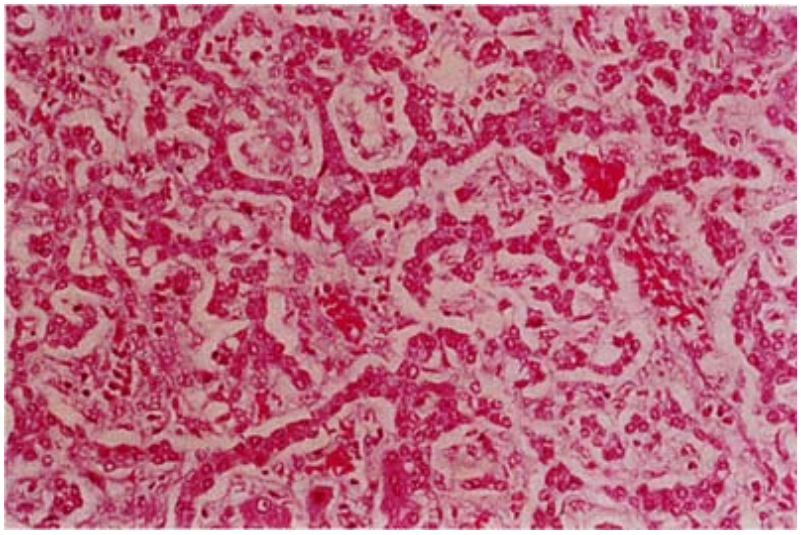
are noted in the syndrome of recurrent jaundice of pregnancy (52).

Cholestatic hepatitis, as noted in the preceding text, is a combined

hepatocellular and cholestatic injury, which may be due to viral hepatitis but which is more often due to drug-induced liver disease (23,50). Acute bile stasis, along with spotty necrosis, hepatocellular ballooning, and/or apoptotic bodies, brings up this differential diagnosis.

Cholestasis with acute cholangitis

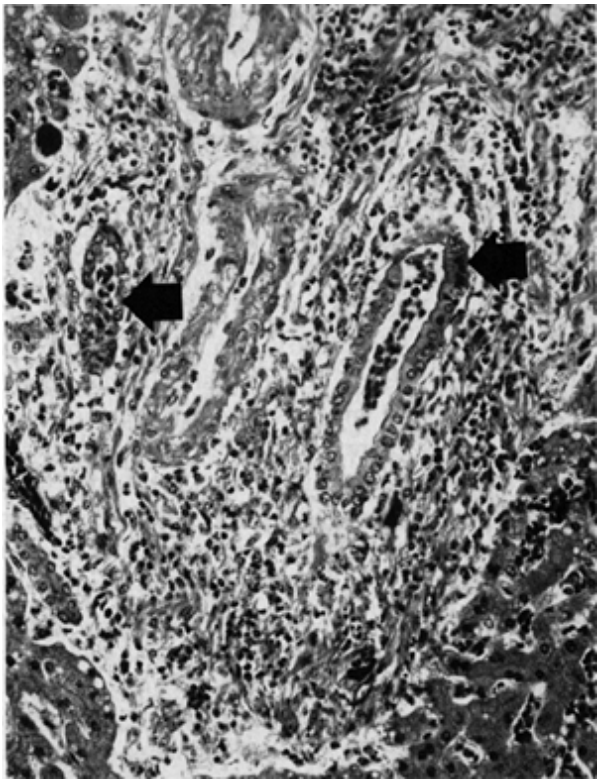
Cholestasis with acute cholangitis is typical of mechanical (large duct) biliary obstruction of any cause, such as choledocholithiasis, neoplasms, strictures (e.g., neoplastic, inflammatory, or postoperative), sclerosing cholangitis, pancreatitis, choledochal cysts, pancreatic pseudocysts, biliary atresia, and several parasitic diseases (e.g., ascariasis, fascioliasis), or even extrinsic pressure from enlarged lymph nodes, tumors, or aneurysms. The key diagnostic feature is acute inflammation (i.e., neutrophils) with epithelial injury of the acinar bile ducts. Bile ducts are usually found adjacent to the corresponding small branches of the hepatic artery with approximately the same diameter. The ducts must be distinguished from ductules or cholangioles, as they have also been called. Reactive ductules ("ductular proliferation") are found at the margins of portal tracts (Fig. 9.46) and should not be confused with bile ducts because changes affecting them do not have the same significance as identical changes in bile ducts (53,54). Ductules, which are usually more angulated than bile ducts, become prominent and appear to proliferate in response to a variety of injuries (55). Neutrophils are commonly situated in and around the reactive ductules, but this does not have the same significance as acute cholangitis, which is characterized by neutrophils in or around the acinar bile duct (Fig. 9.47) and is highly suggestive (but not pathognomonic) of mechanical biliary obstruction. Cholangitis may be present without histologic bile stasis, depending on the degree of obstruction; bile pigment is not usually found without complete obstruction, but acute cholangitis indicates a high likelihood of an obstruction being present. Bile pigment, when present, is seen first in acinar zone 3 and later in zones 2 and 1 as jaundice becomes more profound. Rarely, there is bile in the lumina or epithelium of the acinar bile ducts (Fig. 9.45), but when present, this strongly suggests the presence of obstruction. Other frequent findings include ductular reaction with associated acute inflammation, neutrophilic infiltration of the portal tracts, and bile duct epithelial irregularity or hyperplasia. Severe acute cholangitis is occasionally complicated by rupture, with the development of cholangitic abscesses in the region of the affected bile ducts (Fig. 9.48). Remnants of the disrupted biliary epithelium, bile, and mucin are often located within the abscesses. Xanthomatous cells and foreign body giant cells with phagocytosed bile may also be present. Bile lakes, due to extravasation of bile, and bile infarcts may be seen when there is duct rupture in advanced cases (Fig. 9.49).



▪ **Figure 9.46** Reactive ductules at the margin of an edematous, inflamed portal tract.

Although an acute cholangitis most often denotes extrahepatic biliary tract disease with an ascending infection, there are rare nonobstructive causes,

including toxic shock syndrome, several toxins (e.g., paraquat, methylene diamine, and the toxin of toxic oil syndrome), and a number of drugs (e.g., chlorpromazine, allopurinol and amoxicillin–clavulanate) (56).



▪ **Figure 9.47** Acute suppurative cholangitis in a patient with mechanical biliary obstruction. The portal area is edematous and contains many neutrophils. Neutrophils are present in the lumina of two bile ducts (*arrows*).

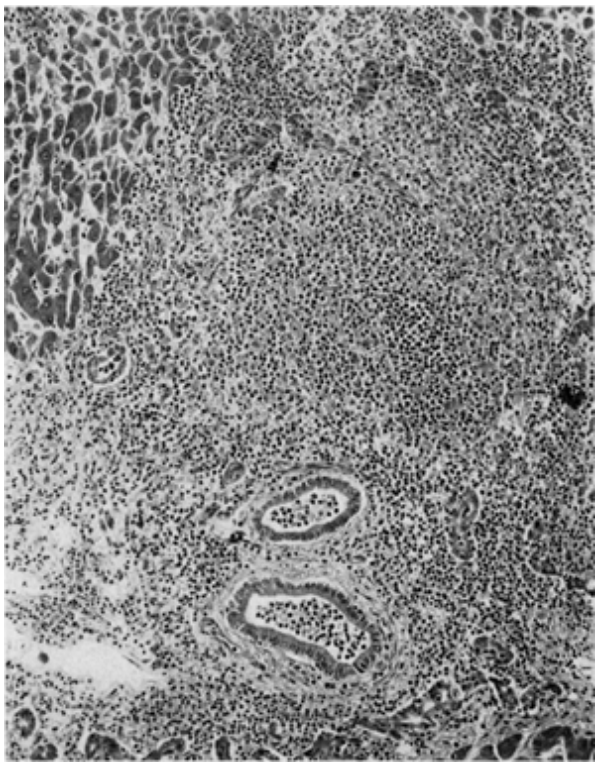
Bile ductular cholestasis

Neutrophils may also be associated with inspissated bile in dilated periportal ductules (Fig. 9.50), a lesion called *bile ductular cholestasis* (57). This lesion is sometimes seen in severely ill patients with sepsis and/or dehydration (57,58), but like other forms of ductular reaction, it does not necessarily indicate mechanical biliary obstruction.

Chronic Cholestasis

Clinical and histologic features of chronic cholestasis appear when there is impaired flow of bile that persists for more than a few weeks. However, most chronic cholestatic disorders are insidious in onset, and chronic cholestasis progresses slowly over the course of years before it becomes clinically apparent. The most reliable histologic sign of chronic cholestasis is the lesion known as *cholate stasis* (53), which is also called *pseudoxanthomatous change, xanthomatous change*, or *feathery degeneration*. These terms refer to a foamy transformation of the cytoplasm of hepatocytes, Kupffer cells, and biliary epithelial cells,

which is seen when there is any type of prolonged (chronic) cholestasis (Fig. 9.51). The affected cells are foamy and often bile stained, as a result of the accumulation of the bile salt and lipid components of bile. Other changes seen in chronic cholestasis include periportal bile pigment, copper accumulation demonstrated with special stains for copper (rhodanine) or by staining for the copper-binding protein, a metallothionein protein within lysosomes (Victoria blue stain) and, in some cases, periportal Mallory bodies. Although there are a number of causes of chronic cholestasis, the most frequent are PBC (see Chapter 24) and primary sclerosing cholangitis (see Chapter 23). Furthermore, in patients who have undergone liver transplantation, allograft rejection can produce bile duct damage and loss, leading to chronic cholestasis.



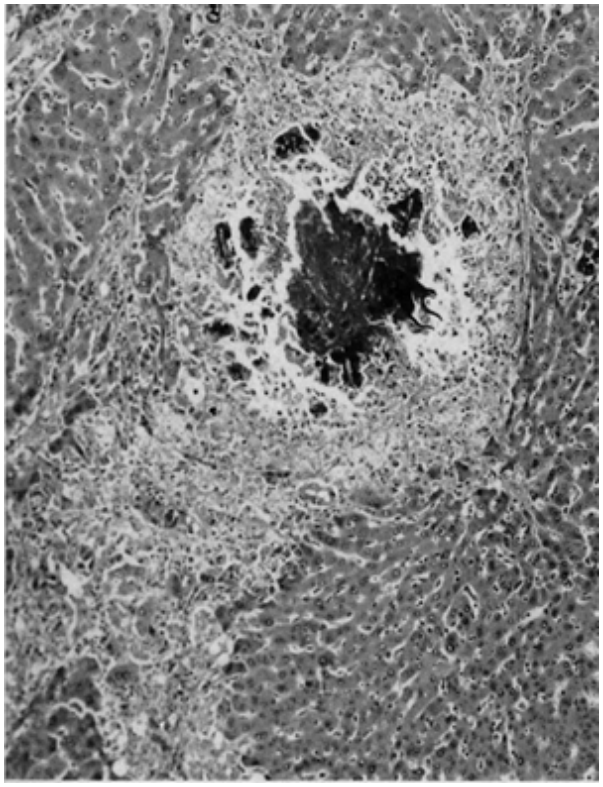
▪ **Figure 9.48** Cholangitic abscess, secondary to ascending cholangitis. The two bile ducts at the bottom of the field are filled with neutrophils.

Primary biliary cirrhosis

The diagnosis of PBC is usually made on the basis of a constellation of clinical, serologic, and histologic findings. In a patient known to have a positive antimitochondrial antibody (AMA) liver biopsy is usually

performed to confirm the diagnosis and assess the stage of disease. In

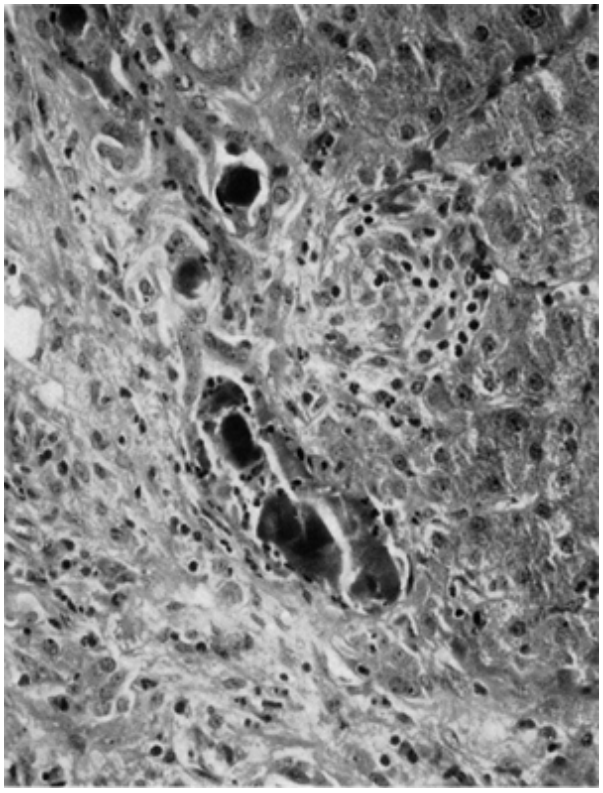
a patient who has not had a complete workup or in whom the AMA is negative, the biopsy may still be diagnostic of the disease.



▪ **Figure 9.49** Bile lake secondary to rupture of a duct and extravasation of bile in mechanical obstruction.

In patients with clinical and laboratory features of chronic cholestasis, particular attention paid to the condition of the acinar bile ducts is critical in histologic evaluation. Ducts affected by PBC show variable chronic inflammation and epithelial injury that lead to destruction of the duct, a lesion called *chronic nonsuppurative destructive cholangitis* (59) or *the florid duct lesion* (60). It is this immunologically mediated destruction of ducts that initiates the disease. Lymphocytes and plasma cells penetrate the basement membrane and insinuate themselves between the epithelial cells, causing destruction of epithelial cells (Fig. 9.52, 9.53) and segments of the basement membrane. Eosinophils and even some neutrophils (despite the fact that it is called *nonsuppurative*) may be present, but it is the lymphocytes that appear to be the primary effectors of the injury. Well-developed lymphoid follicles, sometimes with germinal centers, may be found around or adjacent to the degenerating bile ducts. Epithelioid granulomas (Fig. 9.53), typically less well organized than those of sarcoidosis, are located in the portal areas adjacent to or surrounding the bile ducts, or less often in the

parenchyma, in approximately one third of the cases.



▪ **Figure 9.50** "Bile ductular cholestasis." Periportal ductules are markedly dilated and filled with bile in a patient with bacterial sepsis.

Florid duct lesions are generally considered to be pathognomonic of PBC (53,59,60,61), but they must be distinguished from the hepatitis-associated bile duct lesions (Fig. 9.39) discussed in the preceding text under Chronic Hepatitis. This is most easily accomplished by searching for the features that accompany the destruction of ducts in PBC, namely ductopenia and chronic cholestasis. In chronic hepatitis, loss of ducts is rare and chronic cholestatic features do not develop. The degree of cholangitis in PBC varies greatly from one portal tract to another. Some ducts can appear completely normal, whereas others exhibit striking inflammation and epithelial injury. Therefore, the active diagnostic lesion can be absent in small biopsy samples, and the pathologist is then compelled to apply other criteria and clinical data to the evaluation.

The number of portal tracts lacking acinar bile ducts should be estimated. With the exception of premature infants, a normal liver has a ratio of bile ducts to portal areas of 0.9 or greater (62), usually with the acinar duct running parallel to the hepatic artery branch. In most

patients with PBC, more than half of the portal tracts lack bile ducts, that is, ductopenia (Fig. 9.54), and it is only in the earliest stages that there are no portal areas with missing ducts (62). Also helpful is the frequent presence of periportal bile pigment and cholate stasis (Fig. 9.51).

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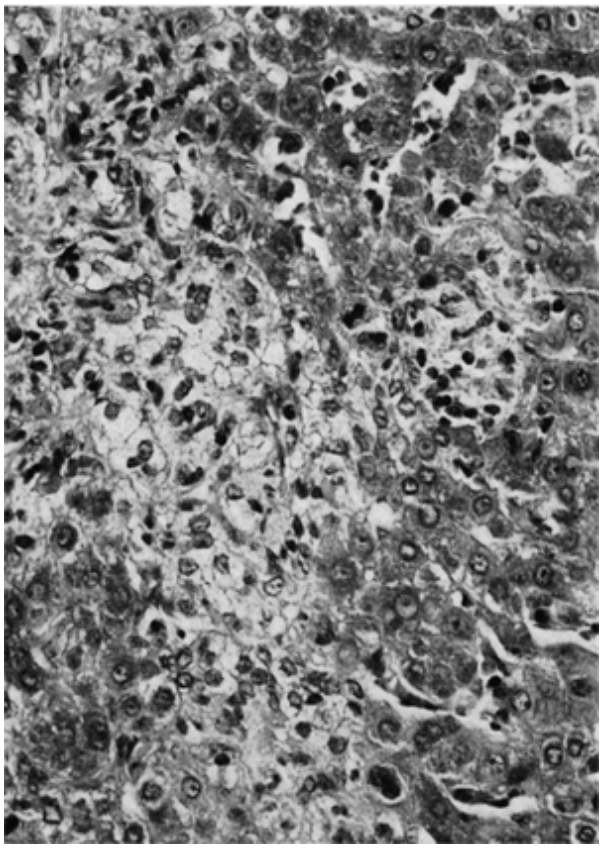
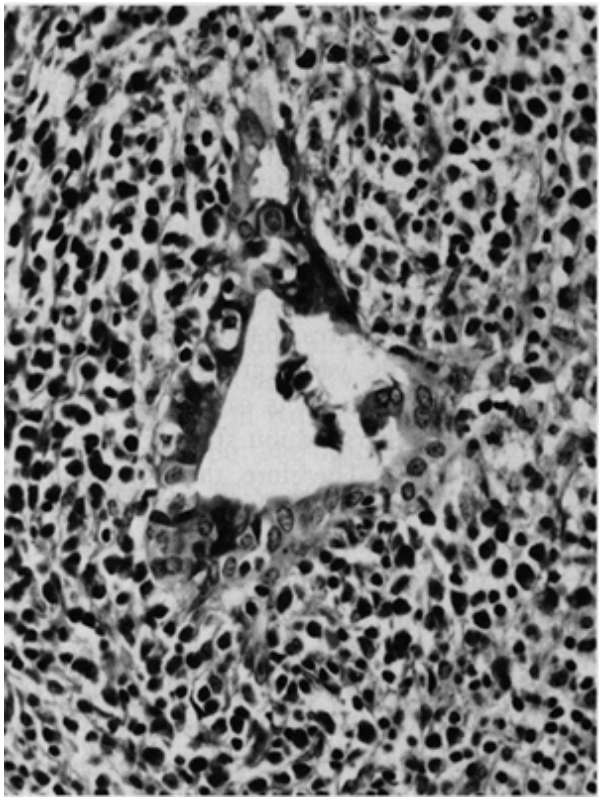
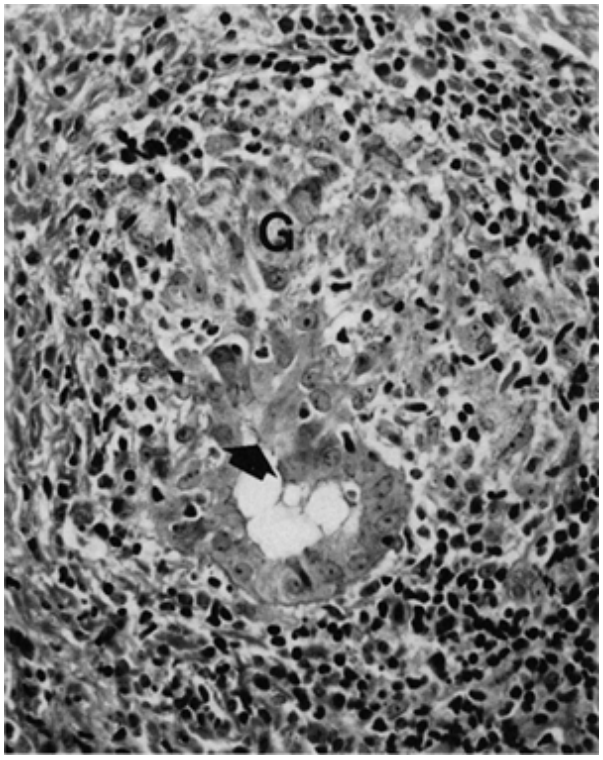
These changes are often subtle and must be sought with care. Small to moderate amounts of copper-binding protein (stainable with Victoria blue) and copper (on rhodanine stain) are frequently detected in hepatocytes in the periportal area (Fig. 9.7). Periportal (zone 1) Mallory bodies, found in 10% to 15% of cases, are further evidence of chronic cholestasis. These are identical to the Mallory bodies of alcoholic and nonalcoholic steatohepatitis except for their location—the Mallory bodies of steatohepatitis are in zone 3.



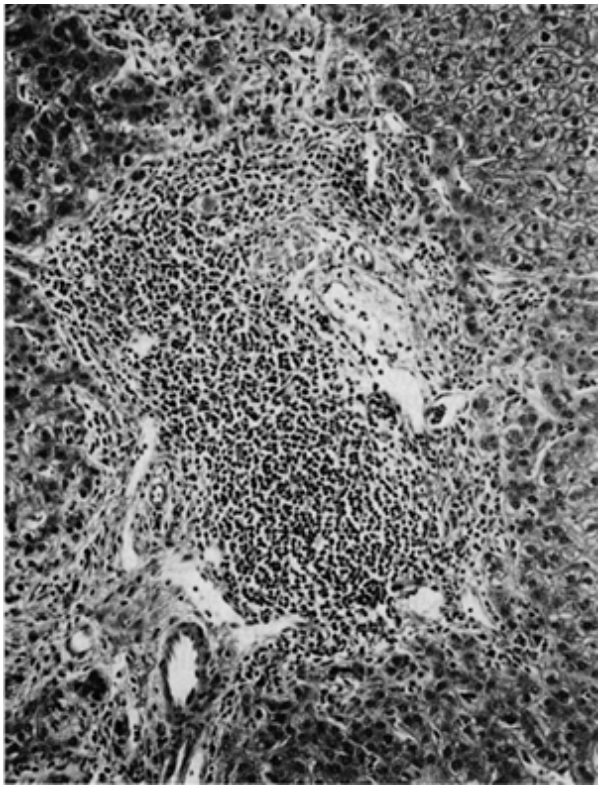
▪ **Figure 9.51** Cholate stasis, indicating chronic cholestasis. The affected cells have pale, foamy cytoplasm because of bile lipid retention.



▪ **Figure 9.52** Florid duct lesion of early primary biliary cirrhosis. The ductal epithelium is infiltrated with inflammatory cells (predominantly lymphocytes), the epithelial cells are severely injured, and the basement membrane is ruptured.



▪ **Figure 9.53** Florid duct lesions of early primary biliary cirrhosis. The duct has ruptured (*arrow*), and there is a poorly formed epithelioid cell granuloma (*G*) adjacent to the damaged duct.

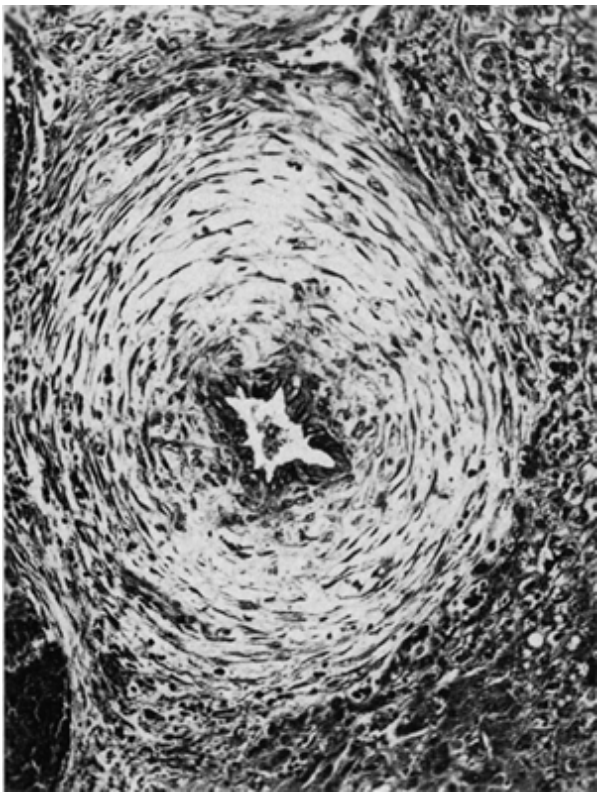


▪ **Figure 9.54** Primary biliary cirrhosis. The portal area lacks a bile duct (ductopenia).

Ductular reaction can be prominent in PBC, particularly in the surrounding portal areas that lack acinar bile ducts. Care must be taken to distinguish the ductules from bile ducts. Hepatocytes are relatively spared, but there is invariably some element of interface hepatitis ("piecemeal necrosis") and hepatitis-like parenchymal injury (61,63). Some investigators have distinguished what they call "biliary piecemeal necrosis," seen in areas of cholate stasis, from "lymphocytic piecemeal necrosis" that is typical of chronic hepatitis (64), but in our experience, these occur together so often that the distinction is not meaningful. There are cases in which the biopsy specimen shows so much hepatocellular injury and interface hepatitis that an overlap syndrome of PBC and autoimmune hepatitis is considered (65,66). In such cases, the clinical and laboratory findings may also suggest both disease processes. However, in our opinion, it is the loss of bile ducts and chronic cholestatic injury that is more significant because bile ducts do not regenerate as readily as hepatocytes and it is chronic cholestasis that leads to cirrhosis in these patients. Therefore, we agree with those who consider the overlap syndrome to be a hepatitic form of PBC (67).

Primary sclerosing cholangitis

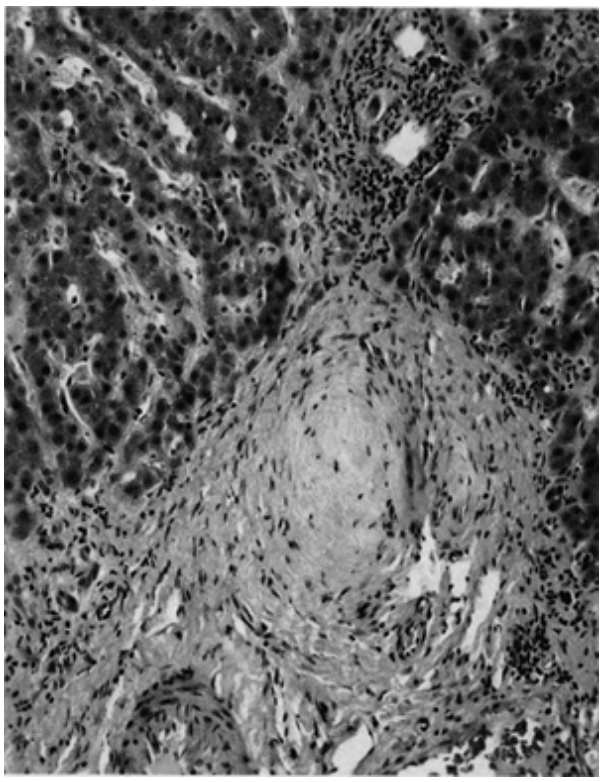
Primary sclerosing cholangitis usually involves the entire biliary tract, but there are occasional cases that affect only extrahepatic or intrahepatic ducts. The extrahepatic ducts are thick and cord-like and have a narrowed lumen. Histologically, a variety of changes may be seen, depending in part on the integrity of the ductal system draining the biopsied area. Changes in the parenchyma are largely due to incomplete chronic mechanical biliary obstruction. Bile pigment is often minimal or absent because obstruction is rarely complete. Clues to the diagnosis can be observed in the portal areas. Some acinar bile ducts may show marked periductal fibrosis with prominent compression and distortion of the epithelium (Fig. 9.55). The epithelium may be almost unidentifiable or even completely atrophic, while a small nodule (cross section of a cord) of fibrous tissue remains in its place (Fig. 9.56). The basement membrane is intact and often thickened. The bile ducts still may be present, but they are often reduced in number or may be totally absent, depending on the stage of the disease. Ductular proliferation is relatively mild compared with that seen in other types of biliary obstruction. Chronic cholestatic features, with cholate stasis and copper accumulation, become increasingly prominent as the disease progresses and may render its distinction from PBC difficult. Furthermore, granulomas, considered a typical feature of PBC, are occasionally present in primary sclerosing cholangitis (68). However, periductal fibrosis is not a typical feature of PBC, while florid duct lesions, in particular the destruction of the basement membrane, are not observed in sclerosing cholangitis.



▪ **Figure 9.55** Primary sclerosing cholangitis. Note the marked periductal fibrosis with compression and atrophy of the epithelium.

Fibrosis follows the loss of bile ducts in both sclerosing cholangitis and PBC, although the mechanism is not clear. In PBC, at least, both ductular proliferation and interface hepatitis, accompanied by collagen deposition, appear to be important (69), but the possible roles of other factors related to chronic cholestasis have not been studied in detail. The fibrosis extends progressively with portal–portal bridging and septum formation, eventually with nodule formation and development of a micronodular biliary cirrhosis indistinguishable from that caused by chronic mechanical obstruction (Fig. 9.57). Staging of disease, if requested, is best accomplished by estimating the degree of fibrosis because any combination of histologic lesions can be found in an individual biopsy specimen. Ludwig

suggested four stages for both PBC (70) and sclerosing cholangitis (71)—stage I (portal), stage II (periportal), stage III (septal), and stage IV (cirrhosis). *Early stage, mid stage*, and *late stage* are also acceptable terms, although they sound less scientific.

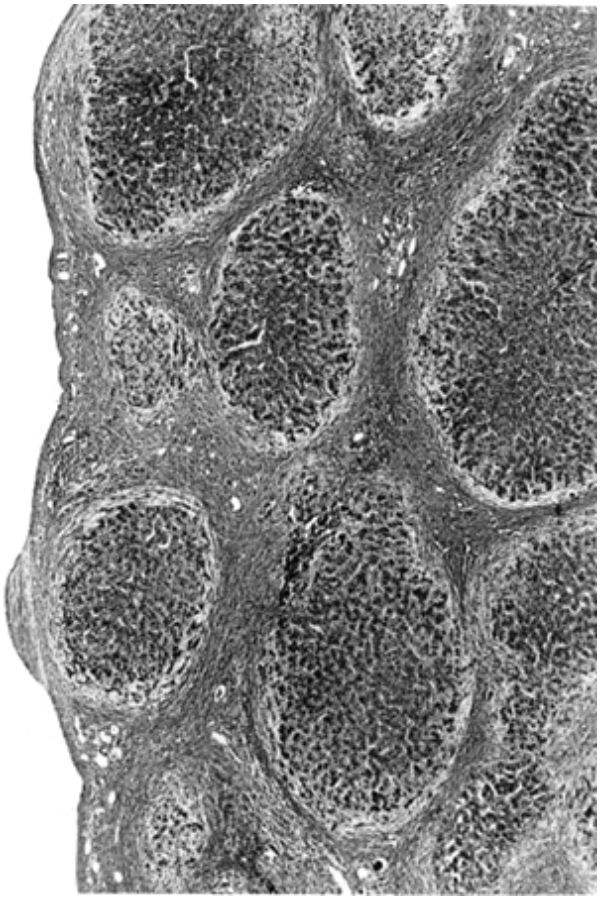


▪ **Figure 9.56** Primary sclerosing cholangitis. The acinar bile duct

is replaced by a fibrous nodule.

Allograft rejection

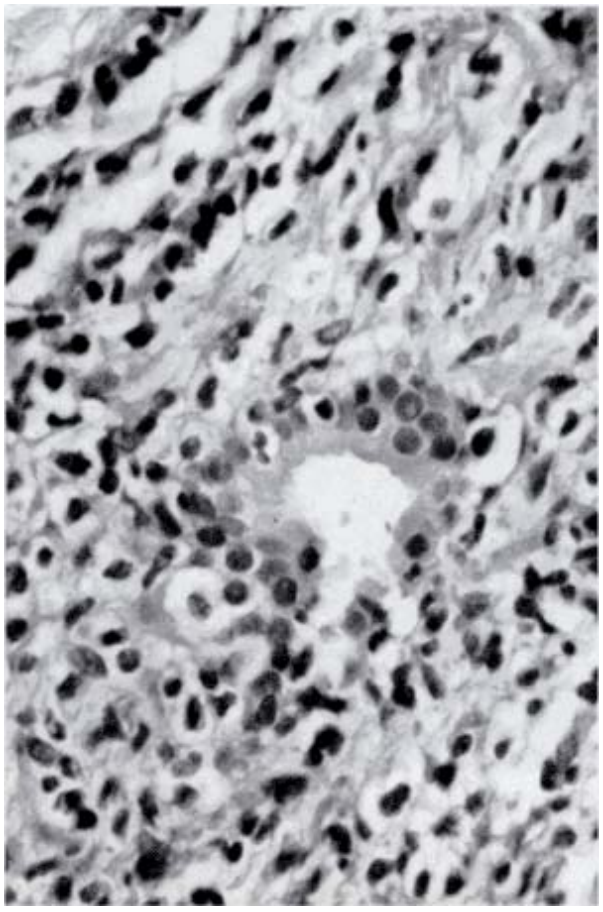
Acute (cellular) rejection is an immunologically mediated attack of the host's defenses against the engrafted liver. The principal targets of the attack are the bile ducts and the endothelium of veins and arteries but not of sinusoids. Snover's triad, consisting of mixed portal inflammation (involving lymphocytes, plasma cells, neutrophils, and eosinophils), bile duct damage (Fig. 9.58) (e.g., rejection cholangitis), and endothelialitis (usually affecting portal vein branches and sometimes the central veins, in the form of lymphocytes attached to the luminal surface of the endothelial cells or between the cell and its basement membrane), is considered diagnostic of rejection (72). These features are variable, and diagnostic findings may or may not present on any individual liver biopsy, so the presence of two of the three features is usually considered sufficient for diagnosis. Cholestasis, hepatocyte ballooning, apoptotic or acidophilic bodies, and focal necrosis may also be present. Cellular rejection may be graded according to the international consensus Banff schema (72), but the clinical utility of this is yet to be proved.



▪ **Figure 9.57** End-stage biliary cirrhosis is typically micronodular with chronic cholestatic features in the residual parenchyma and thick bands of collagen between the nodules, imparting a "jigsaw" pattern to the tissue. Bile ducts are absent.

Chronic (ductopenic) rejection refers to the irreversible damage to the engrafted liver through a combination of immunologically mediated injury and ischemia. It typically follows repeated episodes of acute rejection and so is usually not diagnosed until at least several months after transplantation. Rapidly progressive cases are sometimes seen (acute vanishing bile duct syndrome) but are uncommon. The changes of chronic rejection are thought to be partly due to the injury associated with repeated acute rejection and partly due to reduced arterial flow caused by foam cell arteriopathy in large arteries of the graft. Bile ducts require an arterial blood supply, so the loss of the arteries contributes to the loss of ducts. Changes of chronic rejection include bile duct atrophy and pyknosis, loss of bile ducts (ductopenia) with or without loss of hepatic artery branches, and foam cell arteriopathy in larger arteries, particularly those near the hilum (73). The loss of ducts produces features of chronic cholestasis, and zone 3

fibrosis may also occur because of ischemia.



▪ **Figure 9.58** Acute cellular allograft rejection.

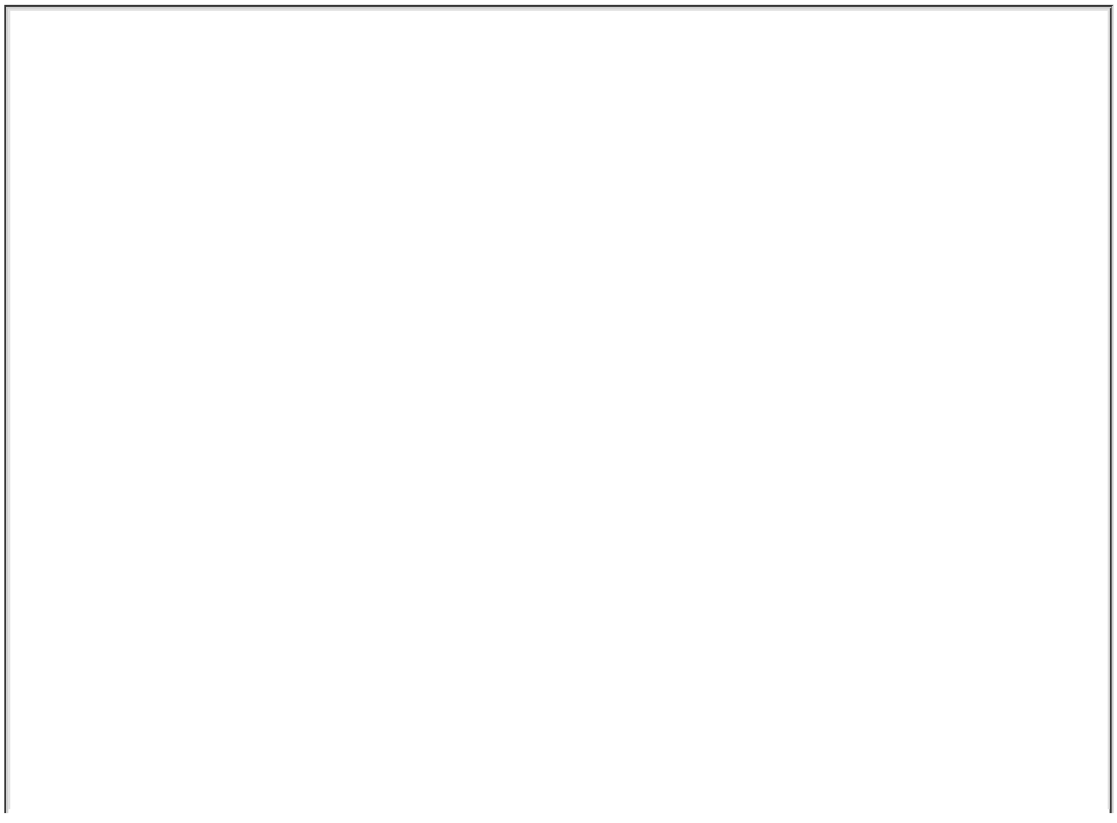
Other chronic cholestatic syndromes

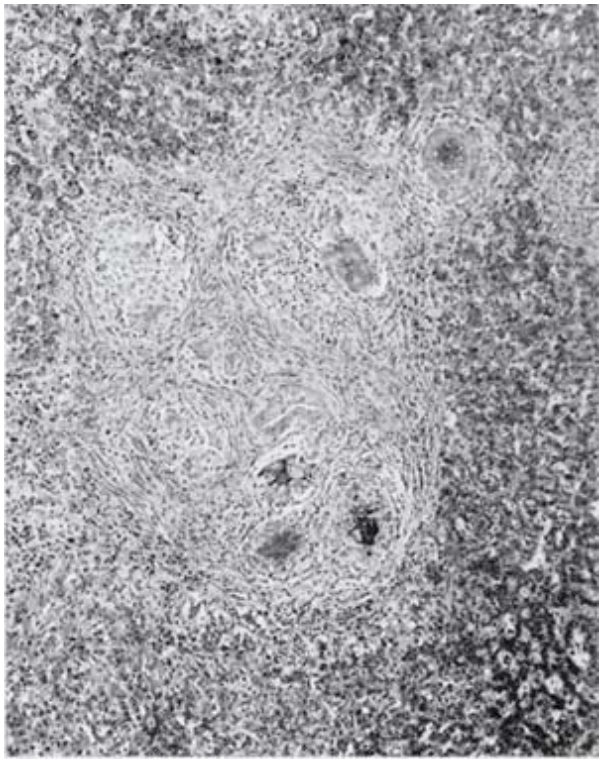
Mechanical obstruction—any of the microscopic changes observed in acute biliary obstruction may be present in biopsy specimens from patients with long-standing obstruction. Additional changes that point to the chronic nature of the process commonly develop when obstruction persists for more than a few weeks. These include periductal sclerosis, cholate stasis, periportal bile stasis, copper accumulation, and sometimes Mallory body formation. Bile becomes inspissated, appearing dark olive green and laminated in sections. Loss of hepatocytes in zone 1 contributes to periportal fibrosis. Cirrhosis may develop when complete or nearly complete obstruction persists for many months, but most patients will be relieved of the obstruction or will develop complications and death before cirrhosis ensues. Biliary cirrhosis is histologically characterized by fibrous septa, linking portal tracts and outlining irregular islands of parenchyma that resemble the

pieces of a jigsaw puzzle (Fig. 9.57).

Extrahepatic biliary atresia in the neonate (see Chapter 47) is more likely to produce secondary biliary cirrhosis than other causes of mechanical obstruction. In this condition, all the morphologic features of acute and chronic biliary obstruction described in the preceding text can be observed, depending on the stage during which a biopsy specimen is obtained. The same criteria for diagnosis of biliary obstruction, described in the preceding text, must be used to differentiate biliary atresia from other cholestatic disorders of the neonate and infant. Some degree of portal fibrosis and ductular proliferation are usually present in biliary atresia and help in distinguishing it from neonatal hepatitis. Diagnostic difficulty may be caused by the presence of giant cell transformation, suggesting hepatocellular injury, in some cases of biliary atresia, but giant cell transformation in neonates should be considered a nonspecific pattern of injury, induced by a variety of hepatic and extrahepatic disorders.

Sarcoidosis sometimes causes a syndrome of chronic intrahepatic cholestasis that can mimic PBC or primary sclerosing cholangitis in many clinical, biochemical, and histologic aspects (72,73,74). In such cases, the liver develops confluent granulomas that destroy bile ducts, cause chronic cholestasis, and may lead to biliary cirrhosis. Although depletion of bile ducts is characteristic, florid duct lesions are uncommon. Granulomatous inflammation in the liver can be found in portal, periportal, and parenchymal areas in the active phase of the syndrome, and the granulomas are better formed and a more dominant feature than those in PBC (Fig. 9.59).





▪ **Figure 9.59** Chronic cholestatic syndrome of sarcoidosis. This portal area has several granulomas and considerable fibrosis but lacks a bile duct.

Secondary sclerosing cholangitis with features nearly identical to primary sclerosing cholangitis may follow mechanical obstruction from a variety of causes, such as surgical manipulation of the biliary tract or tumors of the extrahepatic ducts. Secondary sclerosing cholangitis may also follow chemical injury, such as intra-arterial injection of floxuridine to treat metastatic colon cancer (75) or the injection of formalin into hydatid cysts. Langerhans' cell histiocytosis occasionally affects bile ducts, causing ductal destruction and secondary sclerosing cholangitis (76).

Acquired immunodeficiency syndrome (AIDS) cholangiopathy is a form of secondary sclerosing cholangitis that follows some biliary tract infections such as cryptosporidiosis or CMV in patients with AIDS (77).

Drug-induced chronic cholestasis is an uncommon complication of acute drug injury. A patient with this syndrome develops jaundice, often severe, which does not fully resolve, producing a disorder with some degree of histologic, biochemical, and clinical resemblance to PBC, but not with the insidious onset of the idiopathic forms of chronic cholestasis described in the preceding text. In the early stage there is

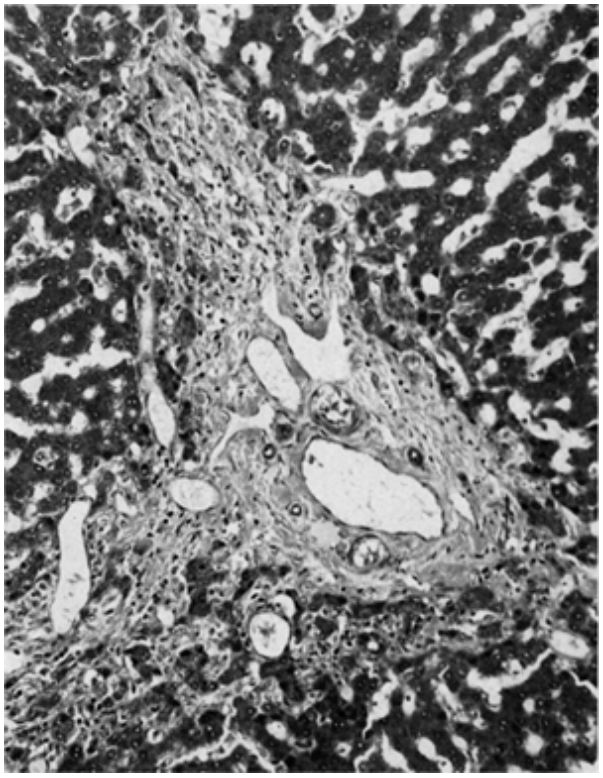
an acute cholangitis, while biopsy specimens in the later stages show ductopenia and chronic cholestatic features (78).

Paucity of intrahepatic bile ducts is a term used for congenital diseases that produce chronic cholestasis associated, as the name implies, with absence of the small acinar ("interlobular") ducts (79). The best defined of these is arteriohepatic dysplasia or Alagille syndrome (see Chapter 47). Bile ducts are present at birth but undergo progressive destruction from early infancy to childhood. In later childhood, there is paucity or absence of acinar bile ducts (Fig. 9.60), paradoxically with mild cholestatic features (80,81). Despite the lack of ducts, it is rare to see bile stasis, and progressive fibrosis or cirrhosis is uncommon. By contrast, children who have bile duct paucity without the other anomalies of Alagille syndrome tend to have a much worse disease, often with progression to end-stage liver disease. Such cases are usually idiopathic but are thought to result from various types of in utero injuries that prevent the normal development of acinar ducts, resulting in a diminished number of ducts at birth.

Idiopathic adulthood ductopenia is a term that is suggested for the rare patient who has a chronic cholestatic syndrome with progressive bile duct loss but does not fit into one of the entities listed in the preceding text (82).

Steatosis (Fatty Liver)

Steatosis can be subclassified into two broad morphologic categories—macrovesicular and microvesicular—on the basis of the size of the fat vacuoles in the liver cells. The distinction is not always sharp, and there are cases where both macrovesicular and microvesicular fat coexist. In general, steatosis is considered macrovesicular when the hepatocytes contain a single large fat vacuole that displaces the nucleus to the edge of the cell, whereas the steatosis is microvesicular when there are numerous small cytoplasmic fat vacuoles that tend to leave the nuclei centrally placed within the hepatocytes. In routinely processed material, the lipid is dissolved by organic solvents, and so frozen sections with special stains (e.g., oil red-O) are necessary to confirm its presence in cases where there is doubt.

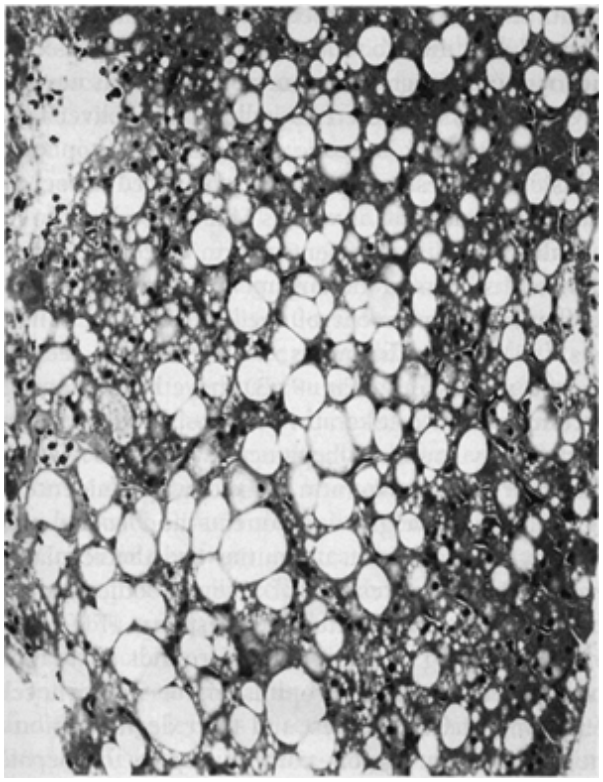


▪ **Figure 9.60** Paucity of intrahepatic ducts (Alagille syndrome). This medium-sized, fibrotic portal area lacks a bile duct, but there is very little inflammation or cholestatic features.

Macrovesicular steatosis is a reaction to a wide variety of injuries, many of which are subclinical and might be more properly regarded as a physiologic adaptation manifested as an imbalance between uptake of lipids from the blood and secretion of lipoproteins by the hepatocyte. Most affected hepatocytes contain a single, medium-sized or large, rounded vacuole that displaces the nucleus and cytoplasm to the periphery of the cell (Fig. 9.61). The vacuoles can be as large as or larger than a normal hepatocyte. Conditions often associated with macrovesicular steatosis include malnutrition, diabetes mellitus, obesity, malabsorption, various debilitating disorders, some metabolic diseases, corticosteroid therapy, and exposure to various other drugs and toxins. Steatosis can be the only change, or it may be associated with other lesions. For example,

in chronic hepatitis C, there is often macrovesicular steatosis associated with the other changes described in the preceding text. Steatohepatitis (alcoholic or nonalcoholic), several metabolic diseases (e.g., Wilson disease), and drug-induced injury (e.g., methotrexate) may be associated with fat accumulation, along with other lesions characteristic of the disease. The location of the fat is quite variable; it is usually

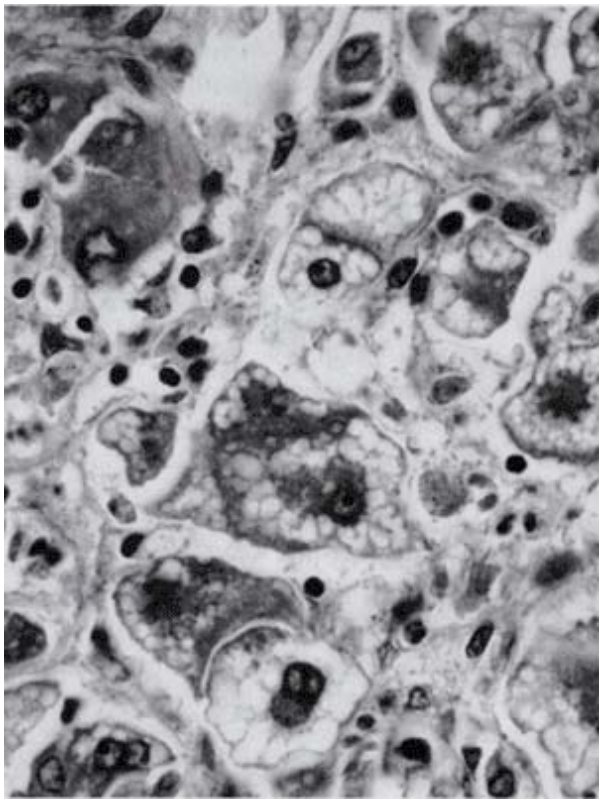
diffuse but can be predominantly in zone 1 or 3.



▪ **Figure 9.61** Macrovesicular steatosis. Most hepatocytes contain a single, large, rounded vacuole that displaces the nucleus and cytoplasm to the periphery of the cell.

Microvesicular steatosis generally connotes a more serious injury than macrovesicular steatosis, although it has been shown that this is a frequent nonspecific finding, especially in autopsy material (83,84). Consequently, a diagnosis of one of the diseases characterized by microvesicular steatosis cannot be made without compatible clinical and laboratory findings. Hepatocytes with microvesicular steatosis show a central nucleus surrounded by sharply defined small vacuoles (Fig. 9.62). Acute fatty liver of pregnancy (85) and Reye's syndrome (86) are well-recognized causes of microvesicular steatosis. A number of metabolic diseases, including fatty acid oxidation disorders, mitochondrial oxidation chain disorders, and urea cycle disorders, are associated with microvesicular steatosis and can mimic Reye's syndrome to varying degrees (87,88). Toxic injury from drugs such as tetracycline, aspirin, valproic acid, antiretroviral nucleoside analogs, and fialuridine can also produce microvesicular steatosis (50,89,90,91). Alcoholic liver injury can also occasionally lead to a toxic microvesicular steatosis, a lesion called *alcoholic foamy degeneration* (92). South American epidemics of hepatitis D and B coinfection are found to have

marked microvesicular steatosis (93) for unknown reasons. Other forms of viral hepatitis, both acute and chronic, may have some degree of microvesicular steatosis, especially if frozen sections and oil red-O stains are used to demonstrate its presence. However, in general fat stains should be reserved for situations in which there is a high clinical suspicion of one of the diseases in which microvesicular steatosis is a cardinal feature, such as acute fatty liver of pregnancy.



▪ **Figure 9.62** Microvesicular steatosis in acute fatty liver of pregnancy. Hepatocytes have a central nucleus surrounded by numerous small fat vacuoles.

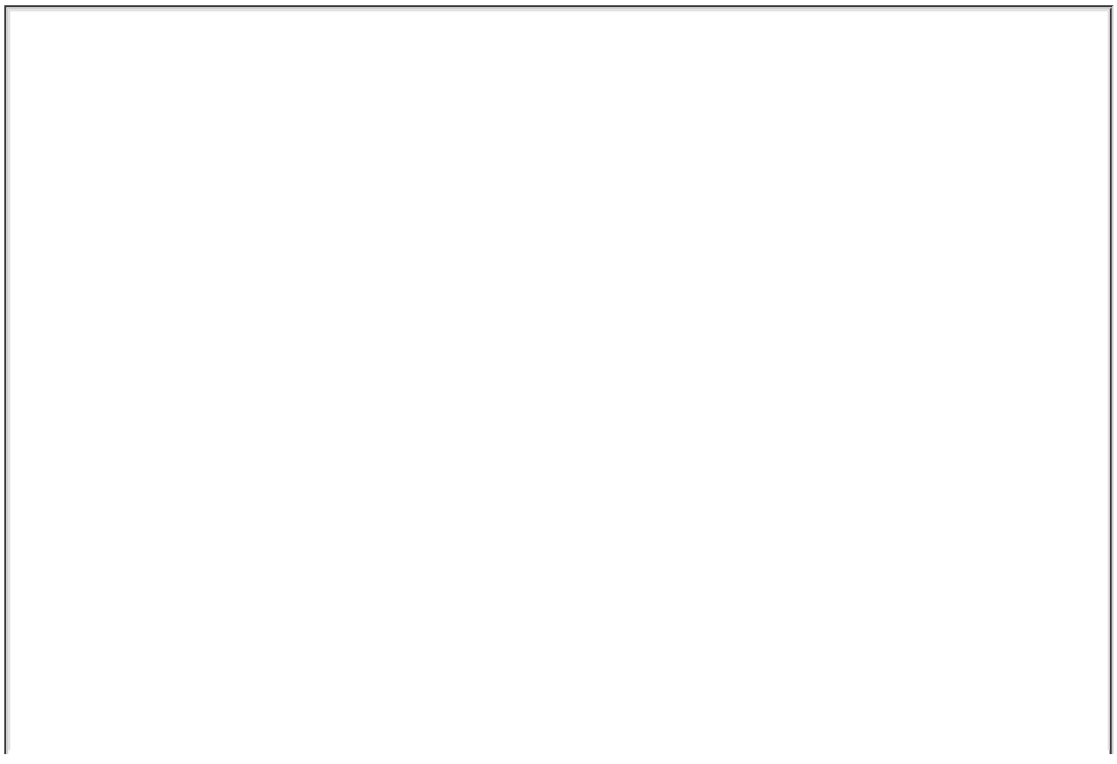
Steatohepatitis: Alcoholic Hepatitis and Nonalcoholic (Metabolic) Steatohepatitis

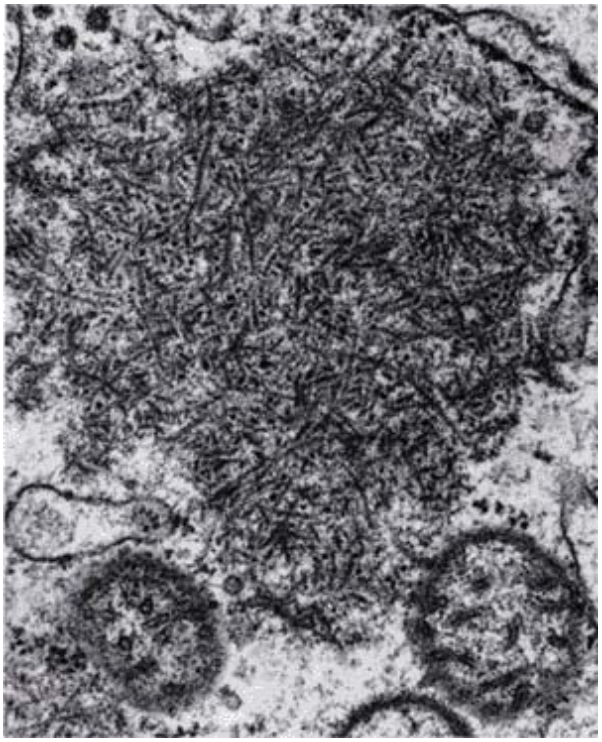
Steatohepatitis

Steatohepatitis is the term used for the morphologic pattern of injury characteristic of the active phase of alcoholic liver disease. Synonyms include alcoholic steatonecrosis, sclerosing hyaline necrosis, and alcoholic hepatitis, when it is found in persons who consume large quantities of alcohol, and fatty liver

hepatitis, metabolic steatohepatitis, and nonalcoholic steatohepatitis, when it occurs in nondrinkers. Because the morphology is so similar, regardless of cause, the term *steatohepatitis* is used here when the lesion is referred to, and alcoholic hepatitis or nonalcoholic steatohepatitis is used for the clinicopathologic entities. Because the pathologist is often unaware of the pertinent clinical information, a diagnosis of steatohepatitis is acceptable until it is known whether the patient consumes alcohol. In patients who are alcoholic, it is presumed that the liver disease represents direct toxicity from ethanol. In those who do not drink, the pathogenesis of the liver disease remains obscure and seems most likely to represent some form of metabolic (probably genetic) disease related to obesity with insulin resistance and/or diabetes (see Chapter 39). It should be emphasized that every liver biopsy specimen with fat and inflammation is not steatohepatitis, despite the name. For example, a patient with preexisting steatosis may have hepatitis-like spotty necrosis and inflammation from an unrecognized drug, undiagnosed virus, or oxidative stress of other unknown cause, but this is not considered steatohepatitis. Only when there are other changes, as described in the subsequent text, is the term *steatohepatitis* appropriate.

Steatohepatitis, whatever the cause, is a chronic lesion that predominantly affects acinar zone 3 (94). Microscopically, this is characterized by a constellation of features that vary in degree and extent from patient to patient. In addition to steatosis (usually macrovesicular but sometimes microvesicular or "mixed"), as noted in the preceding text, there is ballooning of liver cells, most prominently in zone 3. Globular cytoplasmic inclusions, representing enlarged, damaged mitochondria, may be present, as well as Mallory bodies.



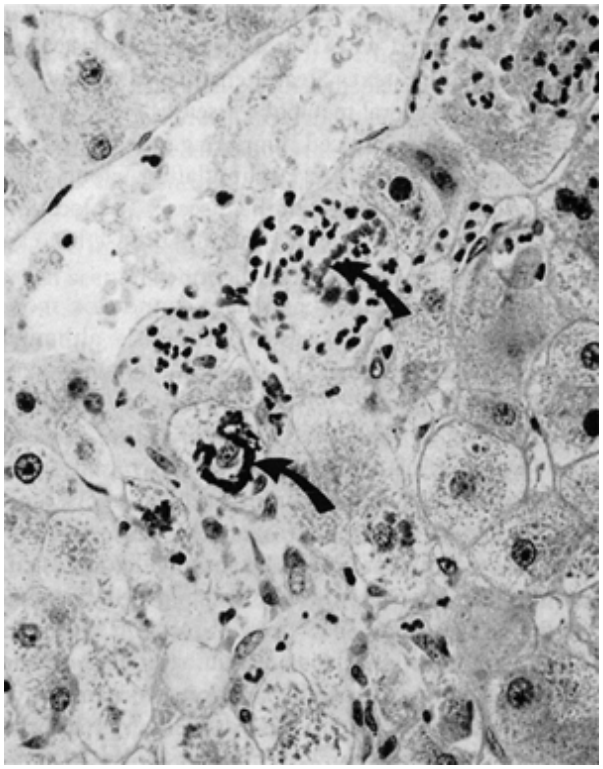


▪ **Figure 9.63** Ultrastructural appearance of a Mallory body. It consists of a tangled mass of intermediate filaments.

Mallory bodies represent a form of cellular injury that results from a derangement of the intermediate filament component of the cytoskeleton of liver cells (95). These filaments, which can be seen by electron microscopy (Fig. 9.63), have been shown to be composed of cytokeratin proteins, both those that are normally found in hepatocytes (types 8 and 18) and other types of keratin, mixed with unidentified high-molecular-weight components and coated with the heat shock protein, ubiquitin, and the regulatory protein p62. The presence of Mallory bodies induces a neutrophilic inflammatory cell response (Fig. 9.64); sometimes a ring of neutrophils surrounds the Mallory body ("satellitosis"). Neutrophils migrate into liver cells containing Mallory bodies, and their degranulation is one of the major factors contributing to the hepatocellular damage (96). Steatosis resolves within 3 to 4 weeks of abstinence from alcohol, while Mallory bodies may take months to disappear. Mallory bodies are

eosinophilic and may be short and irregular or long and rope-like. The cytoplasm around large Mallory bodies is typically empty or rarified (Fig. 9.65), but sometimes it remains eosinophilic and granular (Fig. 9.66), making the Mallory body hard to detect. In mild cases the small Mallory bodies may be few and particularly hard to see (Fig. 9.67), so

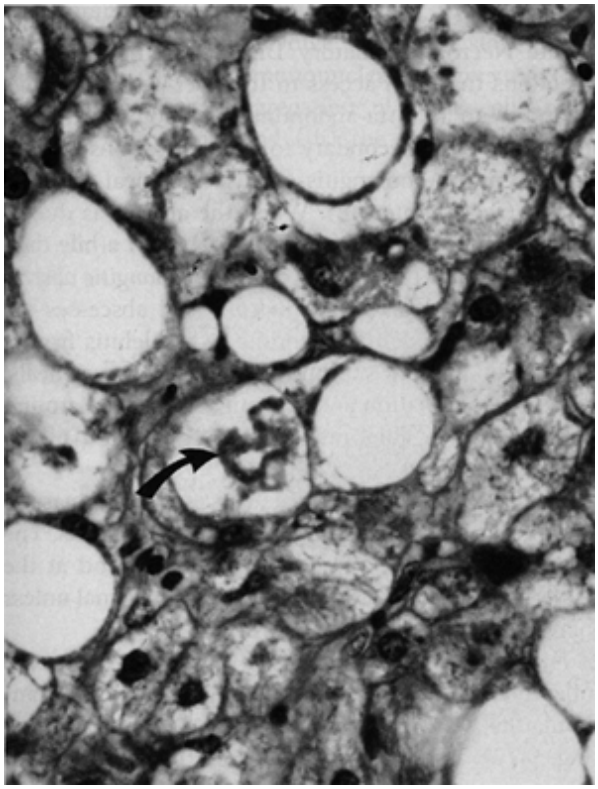
immunostains for ubiquitin (Figs. 9.12, 9.64, 9.68) or p62 protein are particularly helpful in this setting. When Mallory bodies cannot be found despite diligent search, the diagnosis is less certain, but the presence of fat and pericellular fibrosis, described next, strongly suggests steatohepatitis. This is particularly helpful in biopsies from patients with nonalcoholic steatohepatitis, because they tend to have fewer Mallory bodies and less severe active injury than patients with clinical alcoholic hepatitis.



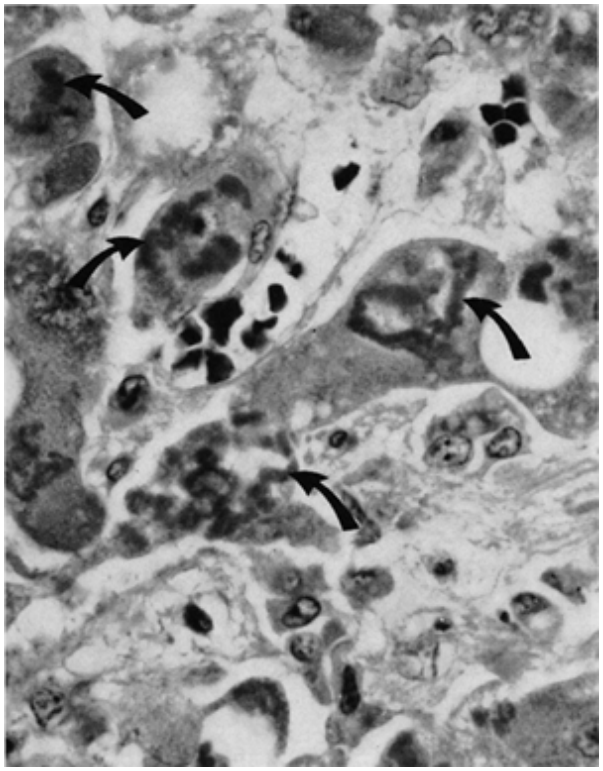
▪ **Figure 9.64** Neutrophilic "satellitosis." In this immunostain for ubiquitin, the dark-staining Mallory bodies (*arrows*) have incited a neutrophilic inflammatory cell response.

Continued activity of steatohepatitis is associated with progressive pericellular fibrosis in acinar zone 3 (Fig. 9.69), with a lattice-like or "chicken-wire" appearance in sections stained with connective tissue stains. Continued scarring also leads to periportal fibrosis and occlusive lesions of terminal hepatic venules (97). With progression of disease, fibrous septa begin to link the chicken-wire fibrosis in zone 3 to extensions of the periportal fibrosis, eventually leading to complete encirclement of islets of hepatic parenchyma. The cirrhosis that develops is usually micronodular (Fig. 9.70), but a macronodular pattern can evolve after alcohol withdrawal. In patients with nonalcoholic steatohepatitis, after cirrhosis develops, the underlying

steatohepatitis may become quiescent with disappearance of fat, active injury, and Mallory bodies, leaving the patient with a histologically cryptogenic cirrhosis (98).



▪ **Figure 9.65** A Mallory body (*arrow*) in a ballooned hepatocyte is easily detected by routine microscopic examination.

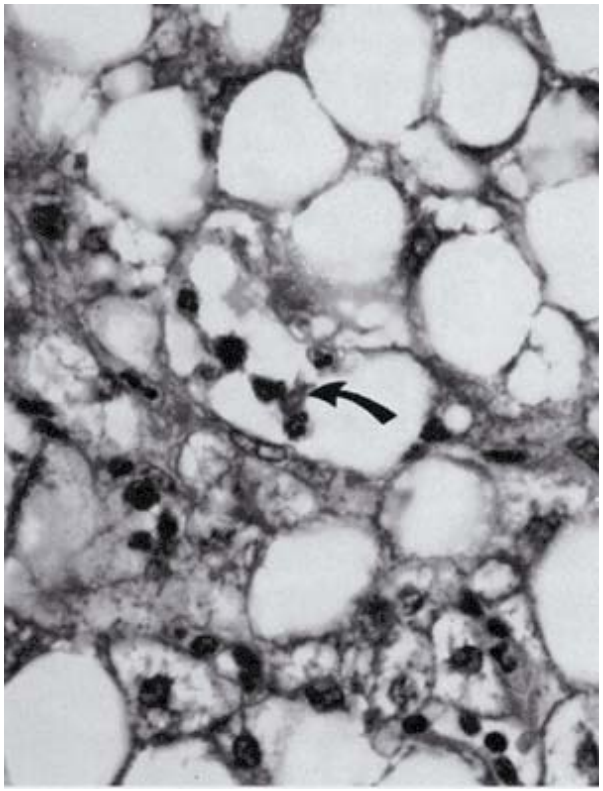


▪ **Figure 9.66** Mallory bodies (*arrows*) in this case of alcoholic hepatitis are less easily seen because the hepatocytes are not as ballooned, but heavy eosin staining brings them out.

Other diseases with features of steatohepatitis

Indian childhood cirrhosis (which occasionally is diagnosed in other countries) is thought to be due to copper toxicity in susceptible children (99). Histologically, the liver shows advanced micronodular cirrhosis with marked copper overload. Fat accumulation is generally mild or absent, but there is considerable hepatocellular injury with ballooning, and Mallory bodies are numerous in many cases.

Drug-induced liver disease from a few drugs may demonstrate Mallory bodies and other features of steatohepatitis (50). Amiodarone and perhexiline maleate are the best characterized of these. Mallory body formation has also been attributed to estrogens, glucocorticoids, calcium channel blockers, and antiretroviral drugs, but the evidence for these is less convincing.



▪ **Figure 9.67** Mallory bodies (*arrow*) in a case of mild nonalcoholic steatohepatitis. The Mallory bodies in this case are all small and thin, making them difficult to find. Note the two neutrophils adjacent to the Mallory body.

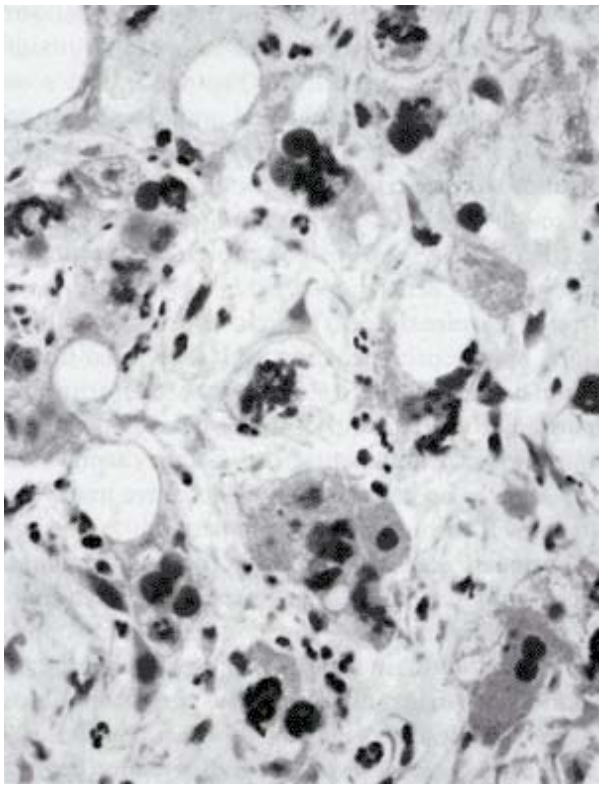
After jejunoileal bypass surgery, some patients developed severe steatohepatitis (100), leading to death from hepatic failure in a few cases. Similarly, steatohepatitis has been reported occasionally in patients with postsurgical short gut syndrome and gastroplasty.

Other diseases with Mallory bodies include chronic cholestatic syndromes such as PBC and primary sclerosing cholangitis, although in these diseases the Mallory bodies are in zone 1 rather than zone 3 and other features of steatohepatitis are lacking. Wilson disease may have Mallory bodies in the cirrhotic stage and can have steatosis as well, making it difficult to distinguish this disease from steatohepatitis. Finally, tumors of hepatocellular origin, including hepatocellular carcinoma, hepatocellular adenoma, and occasionally, focal nodular hyperplasia may contain Mallory bodies in the tumor cells.

Granulomatous and Suppurative Diseases

Space-occupying inflammatory lesions

Abscess is the term used for a collection of neutrophils (i.e., "pus" or purulent inflammation) in a confined space. A microscopic focus of neutrophils may be termed a *microabscess*. This is the typical lesion of some disseminated infections such as listeriosis and salmonellosis, as discussed in the preceding text under "Acute Necroinflammatory Disease". Other bacterial infections that gain access to the liver—blood borne, secondary to an intra-abdominal infection, or through the biliary tract, secondary to mechanical obstruction and ascending cholangitis—cause a typical *pyogenic abscess* (see Chapter 48). When the abscess is due to ascending cholangitis and the remnants of a bile duct can be found in the lesion, the term *cholangitic abscess* (Fig. 9.48) is appropriate. *Pylephlebitic* abscesses are secondary to an acute ascending pylephlebitis from a focus of abdominal suppuration. As an abscess heals, chronic inflammation and scarring can be seen around the edges, with compression and destruction of the hepatic parenchyma.

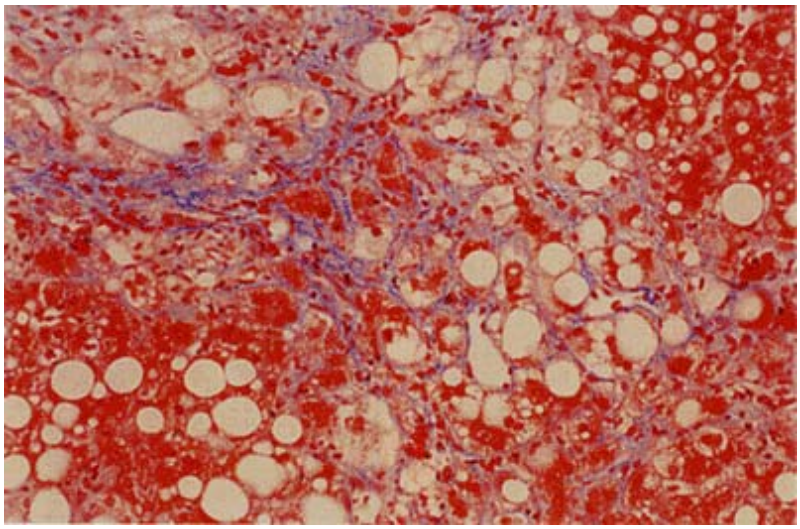


▪ **Figure 9.68** Numerous Mallory bodies in a case of alcoholic hepatitis are easily seen with the immunostain for ubiquitin.

Amebic abscess is not a true abscess. It is a mass of amorphous, necrotic tissue infected with amebas. The amebic trophozoites (Fig.

9.71) can be found at the edges of the lesion, but inflammation is minimal unless there is bacterial superinfection.

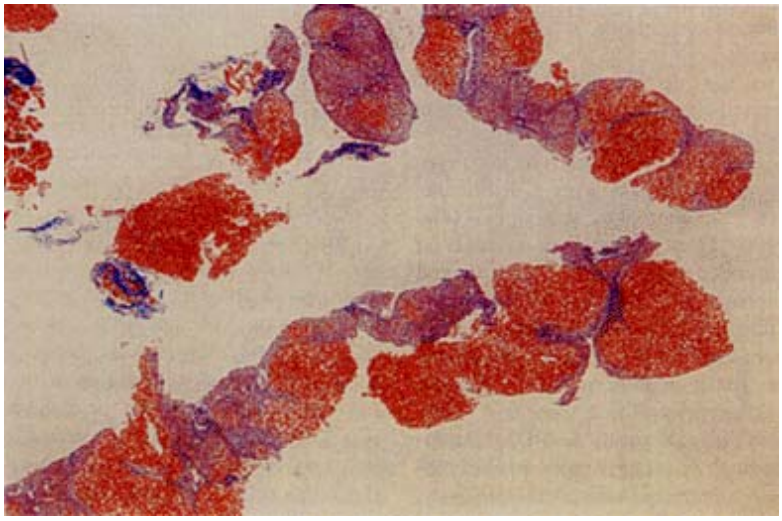
Inflammatory pseudotumor (101) is a mass of chronic inflammatory cells (in particular plasma cells), xanthomatous histiocytes, myofibroblasts, and fibroblasts (Fig. 9.72). Its pathogenesis is uncertain, but at least some cases result from healing abscesses. Some cases are suspected to be true neoplasms, and the term *inflammatory myofibroblastic* tumor is used.



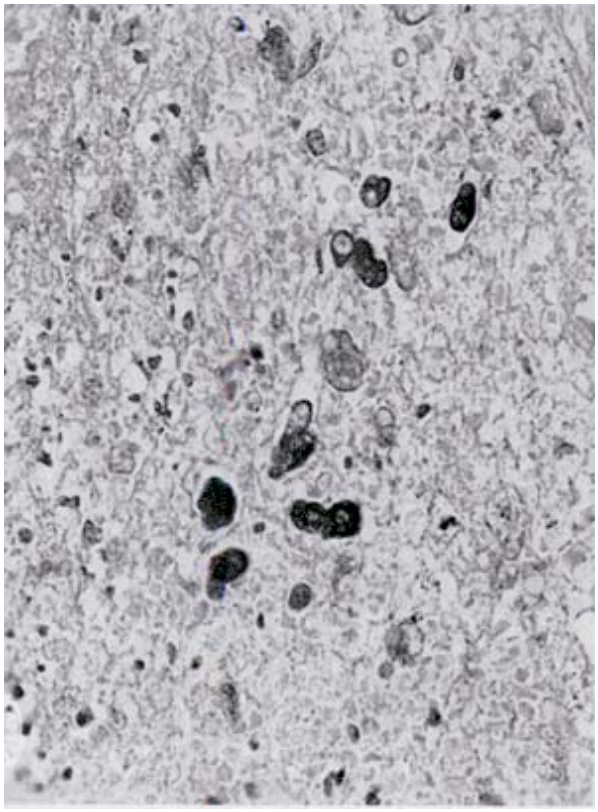
▪ **Figure 9.69** Steatohepatitis with early pericellular ("chicken-wire") fibrosis in acinar zone 3, best appreciated with the Masson stain.

Granuloma is a compact, organized collection of mature mononuclear phagocytes (Fig. 9.73) that may or may not be accompanied by accessory features, such as other types of inflammatory cells, necrosis, or scarring (102,103,104). Granulomatous inflammation is a response to an injury that cannot be contained and eliminated by the usual acute inflammatory response. Granulomas evolve in three stages: (i) An infiltrate of young mononuclear phagocytes, (ii) the maturation and aggregation of these cells into a mature granuloma, and (iii) the potential further maturation of these cells into an epithelioid granuloma (102). A small focus of granulomatous inflammation, consisting of only a few epithelioidhistiocytes, is often called a *granulomatoid focus*. The term *granulomatous hepatitis* should be reserved for cases in which there are both granulomas and necroinflammatory hepatocellular injury, as discussed in the preceding text under Necroinflammatory Diseases. The many causes of hepatic granulomas are discussed in Chapter 52. In the broadest sense, granulomas can be classified as infectious or

noninfectious.



▪ **Figure 9.70** Alcoholic micronodular cirrhosis in a needle biopsy.



▪ **Figure 9.71** Amebic trophozoites (dark-staining) are easily seen with the PAS stain. They are present in the amorphous, necrotic

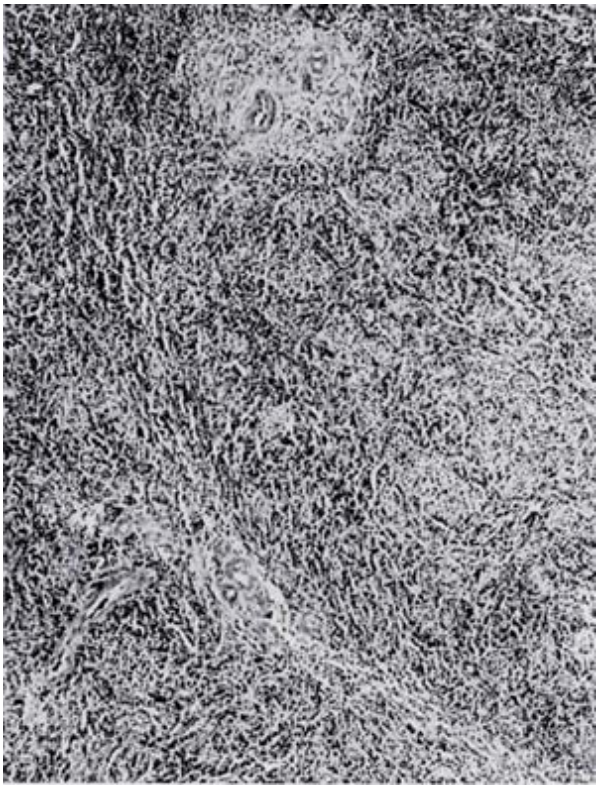
tissue at the edge of an amebic abscess.

Infectious granulomas

Infectious granulomas may be due to any class of organism, and these can sometimes be identified in the tissue or there may be other features to provide a clue to the diagnosis:

Viruses. Granulomatoid foci or, rarely, true granulomas may be seen in the liver in some viral infections, such as infectious mononucleosis and CMV mononucleosis. Other features of mononucleosis hepatitis are invariably present.

Rickettsia. Q fever (*Coxiella burnetii* infection) typically produces granulomas with a distinctive, although not pathognomonic, appearance (Fig. 9.74) (105,106). These lesions have a central fat vacuole surrounded by epithelioid histiocytes and other inflammatory cells. Brightly eosinophilic strands of fibrin form a ring within the granuloma, so these lesions are called *fibrin ring granulomas*. Similar granulomas are described occasionally in patients with a number of other diseases (CMV, EBV, hepatitis A, AIDS, boutonneuse fever, staphylococcal sepsis, toxoplasmosis, visceral leishmaniasis, allopurinol toxicity, giant cell arteritis, Hodgkin's disease, and lupus erythematosus) (103). In each case these are unusual manifestations of the diseases, whereas the fibrin ring granulomas are typical of Q fever hepatitis. The organisms cannot be identified in tissue, but finding fibrin ring granulomas should prompt serologic testing for *Coxiella burnetii*.



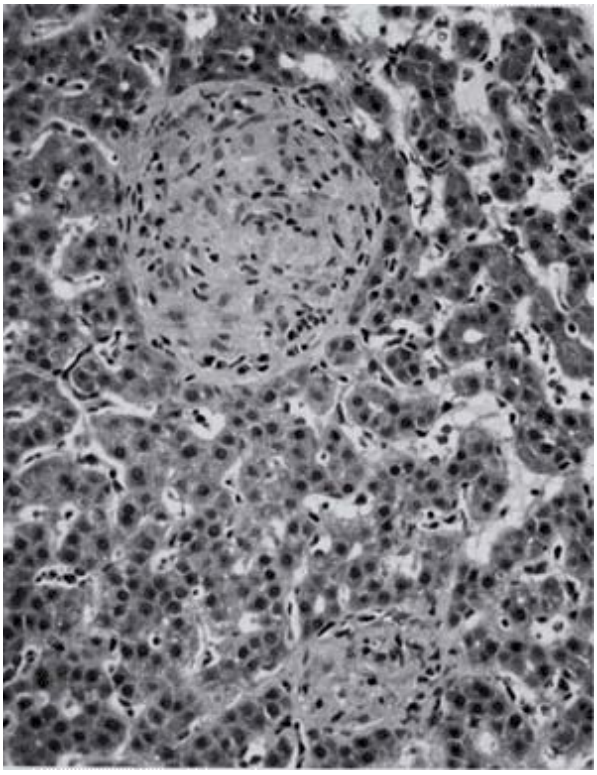
▪ **Figure 9.72** Inflammatory pseudotumor is a mass of inflammatory cells, histiocytes and fibroblasts, presumably the result of a healing inflammatory lesion.

Bacteria. True granulomas are unusual in most bacterial diseases except in brucellosis and occasionally in syphilis; organisms are almost never demonstrable. Microabscesses or ill-formed granulomas that contain neutrophils suggest a bacterial infection such as catscratch disease, melioidosis, tularemia, or typhoid.

Mycobacteria. Caseous necrosis (Fig. 9.75) should suggest miliary tuberculosis, although acid-fast bacilli may be difficult or impossible to find. Absence of caseation, of course, does not exclude tuberculosis. Lepra bacilli are difficult to find in granulomas in tuberculoid leprosy, but they can be demonstrated in large numbers with special stains in untreated lepromatous leprosy in enlarged reticuloendothelial cells that have a foamy cytoplasm (lepra cells) and are clustered in granuloma-like formations (107). Similarly, patients with AIDS

and disseminated *Mycobacterium avium intracellulare* frequently have hepatic involvement with "macrophagic" granulomas composed of hypertrophied, grey–blue macrophages containing hundreds of acid-fast bacilli (Fig. 9.76) (108). Giant cells and

inflammatory cells are generally absent, as is caseous necrosis.



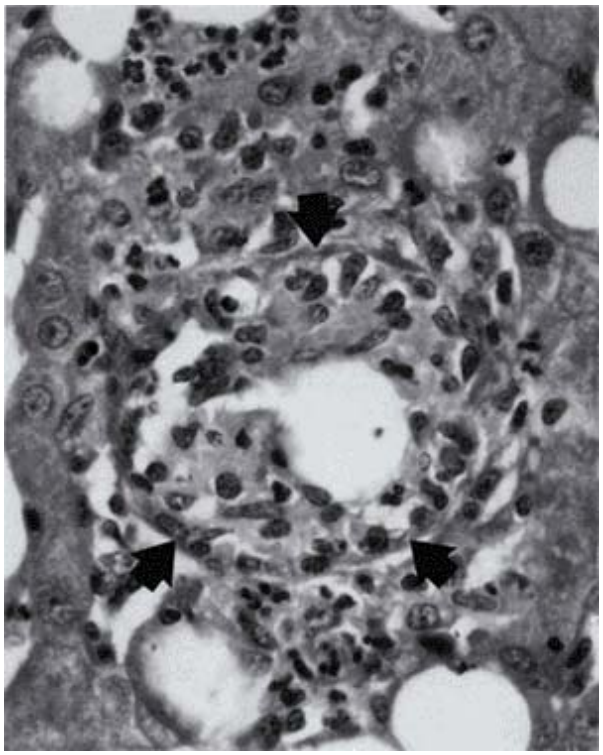
▪ **Figure 9.73** Typical noncaseating granulomas in a patient with sarcoidosis. The granulomas are composed predominantly of epithelioid histiocytes. The smaller granuloma in the lower part of the field probably represents a tangential cut through a larger lesion.

Fungi. Granulomas in the systemic mycoses often contain the fungal spores or hyphae that may be visible with hematoxylin and eosin but are best demonstrated with the Gomori methenamine silver stain (Fig. 9.77).

Protozoa. Organisms in visceral leishmaniasis are usually found in hypertrophied Kupffer cells, but granulomas may be seen, sometimes with central necrosis and sometimes with fibrin rings (103).

Parasites. Several parasitic diseases can involve the liver with a granulomatous response (103). By far the most important of these is schistosomiasis. The granulomas in this disease usually contain intact eggs (Fig. 9.78) or their chitinous remnants. The granulomas in the same biopsy specimen can be of differing ages, from "active" granulomas with many epithelioid cells and eosinophils to round scars containing fragments of egg chitin.

Granular black schistosomal pigment, which is the acid hematin residue from breakdown of host hemoglobin by the parasite, is usually readily identified in the reticuloendothelial cells in livers harboring active granulomas. Other parasitic diseases in which eggs can be found in association with a granulomatous response include hepatic capillariasis, fascioliasis, paragonimiasis, and ascariasis. Visceral larva migrans, usually attributable to the larvae of *Toxocara* species, produces a characteristic lesion in the liver. The granulomas are associated with a massive outpouring of eosinophils and often reveal areas of central necrosis resulting from degeneration and degranulation of eosinophils (Fig. 9.79); there can be Charcot-Leyden crystals in the necrotic foci, but larvae are only rarely identified (109).



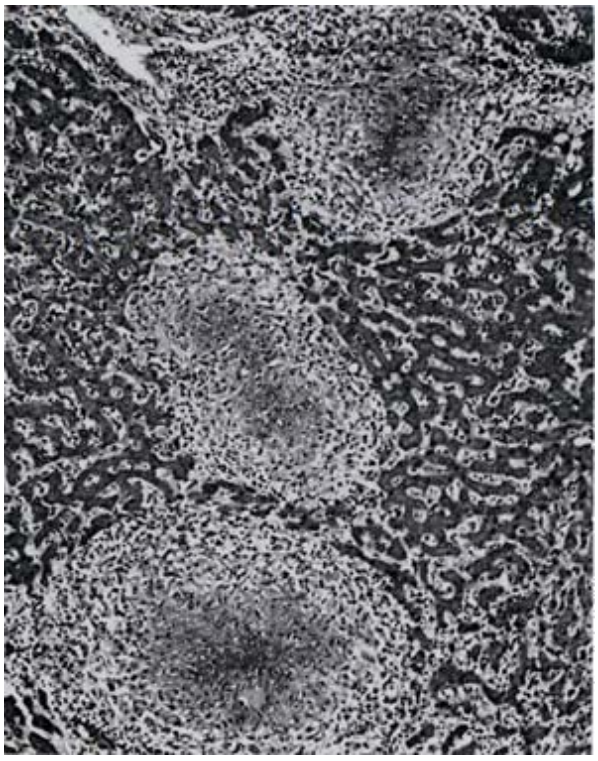
▪ **Figure 9.74** Fibrin ring granuloma in Q fever (*Coxiella burnetti* infection) has a central fat globule surrounded by epithelioid cells and a brightly eosinophilic ring of fibrin (*arrows*).

Noninfectious granulomas

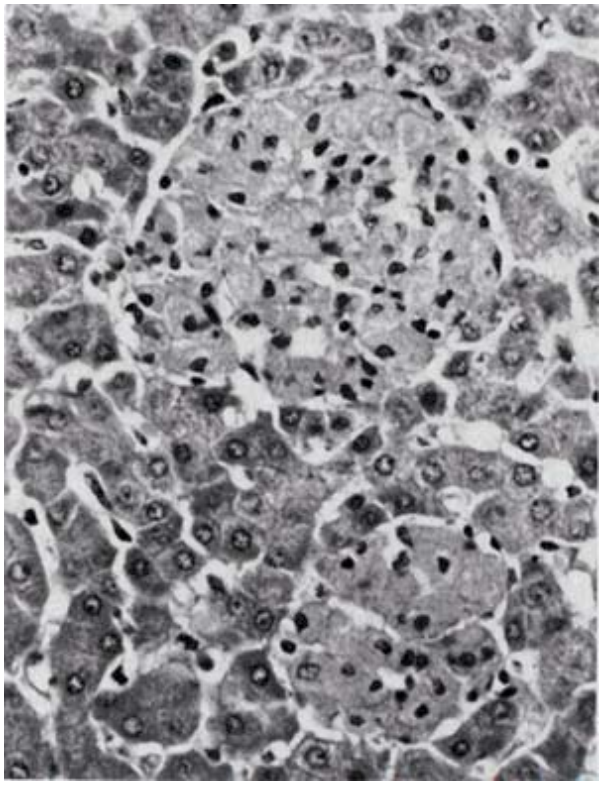
Sarcoidosis is the prototype of all granulomatous diseases. It is always a diagnosis of exclusion, requiring demonstration of granulomas in two or more tissues with exclusion of all known causes of granulomatous disease. At least 90% of patients with sarcoidosis have hepatic

involvement, although in most it is clinically insignificant. Granulomas in sarcoidosis have no specific identifying features, but they do tend to have certain characteristics. The granulomas are scattered

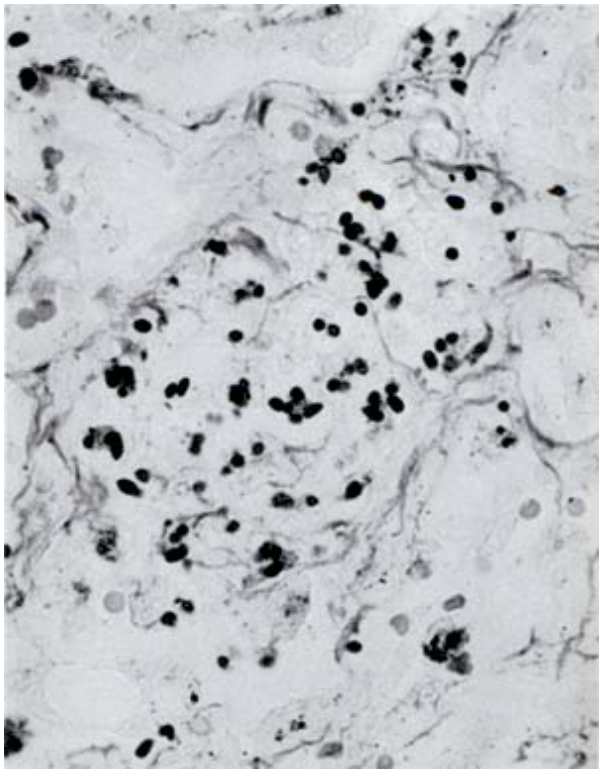
throughout the liver tissue, but most tend to be portal or periportal. Granulomas of all ages are typically present. The earliest lesions consist of small, loosely arranged clusters of a few epithelioid cells within the acini. Older lesions are globular or ovoid and sharply defined (Fig. 9.73), and in cases with severe involvement, the granulomas may be confluent. Young granulomas tend to be composed predominantly of epithelioid cells, often with a few lymphocytes, while giant cells are a sign of aging. The giant cells may contain asteroid bodies (Fig. 9.80), Schaumann bodies, or calcium oxalate crystals. Older granulomas often have many giant cells, and sometimes, when the granulomas have resolved, a few naked giant cells may remain in the tissue. Usually, however, sarcoid granulomas heal by scarring, and often there is a rim of dense collagen around each granuloma (Fig. 9.81). The last remnant is a fibrous nodule, sometimes containing one or two giant cells. The granulomas in sarcoidosis are typically noncaseating, but rarely caseous necrosis may be seen in otherwise typical cases (74).



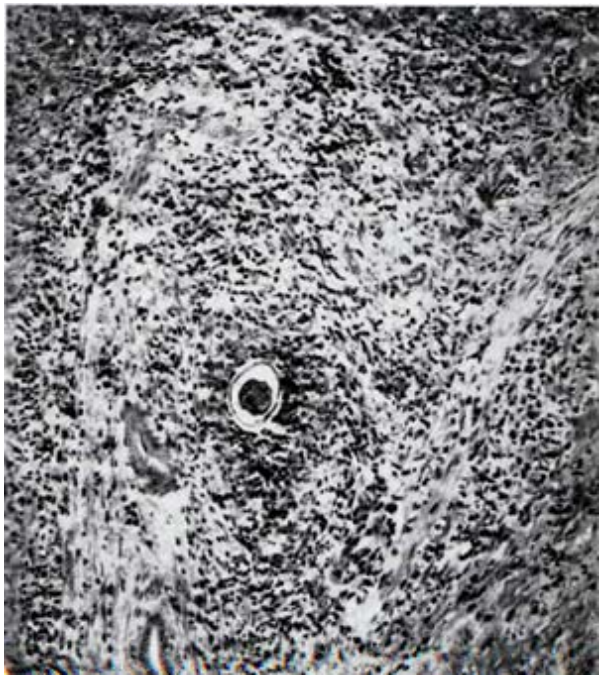
▪ **Figure 9.75** Miliary tuberculosis with amorphous, caseous necrosis in the centers of the granulomas.



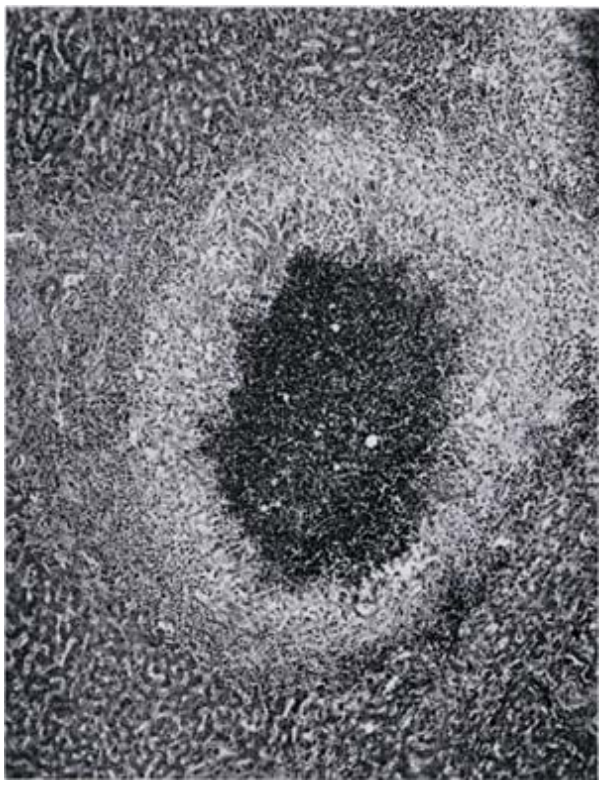
▪ **Figure 9.76** Disseminated *Mycobacterium avium intracellulare* in a patient with acquired immunodeficiency syndrome. The liver contains "macrophagic" granulomas composed of hypertrophied, grey–blue macrophages. Acid-fast stains typically show hundreds of acid-fast bacilli.



▪ **Figure 9.77** Gomori methenamine silver stain is useful for demonstrating fungal organisms in systemic mycoses. Yeast forms can be seen in this case of histoplasmosis.

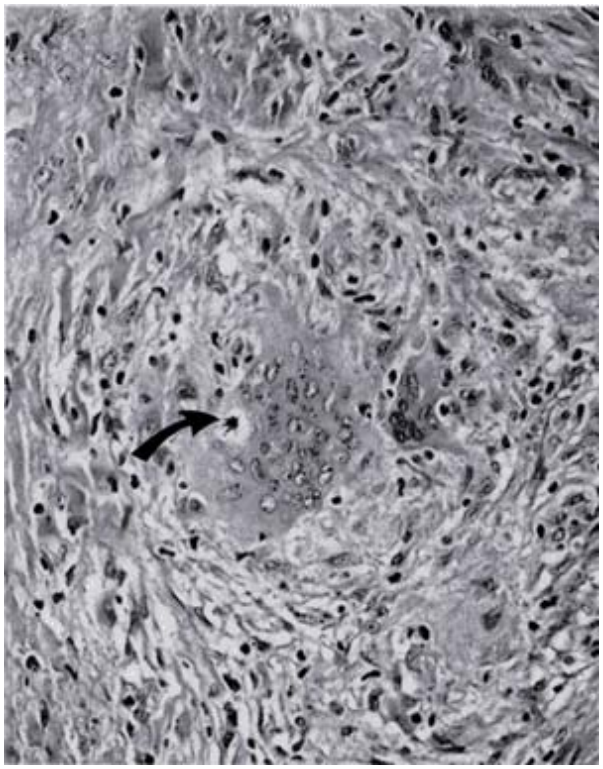


▪ **Figure 9.78** Schistosomiasis. This portal granuloma contains an embryonated egg.



▪ **Figure 9.79** Visceral larva migrans. This eosinophilic granuloma consists of a central area of necrotic eosinophils surrounded by a palisade of epithelioid histiocytes and an outer zone that contains many additional eosinophils.

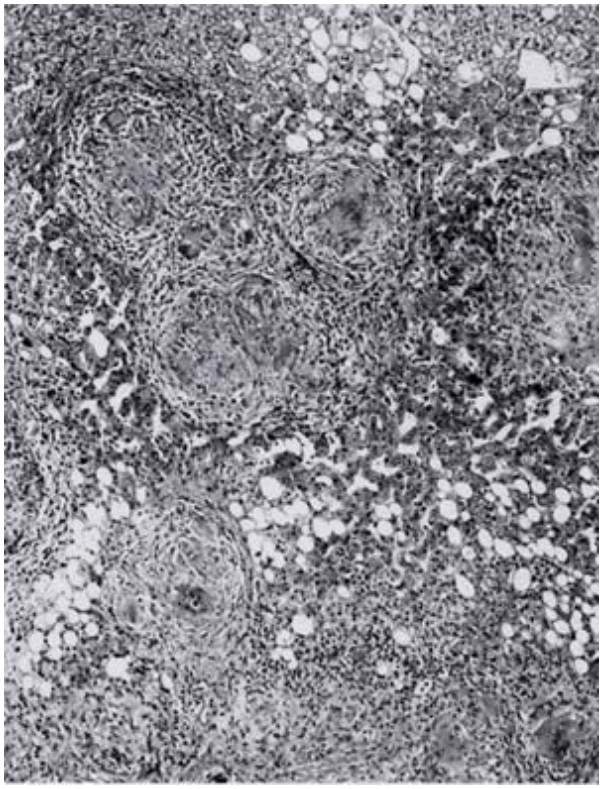
In most patients with sarcoidosis, the hepatic granulomas are clinically silent. An unknown proportion of patients, however, come to clinical attention because of clinical symptoms and signs of cholestatic liver disease, portal hypertension, or abnormal liver test results. Biopsy results in such cases show a spectrum of changes (74). Some patients have only sarcoid granulomas without other associated changes, but the majority show some degree of associated necroinflammatory injury (e.g., apoptosis, focal necrosis, chronic portal inflammation), features of chronic cholestasis (e.g., cholate stasis, bile duct loss), or some combination of these. Extensive portal fibrosis may cause severe bile duct loss leading to a biliary cirrhosis, or there may be fibrous obliteration of portal vein branches, producing portal hypertension.



▪ **Figure 9.80** Sarcoidosis. An old fibrotic granuloma containing several giant cells, one of which has an asteroid body (*arrow*).

In a significant proportion of patients a cause is never found for the sarcoid-like hepatic granulomas, and no extrahepatic granulomas are found to confirm the diagnosis of sarcoidosis. Nevertheless, such cases probably represent the same idiopathic disease, but until the cause of sarcoidosis is discovered, these cases will remain undiagnosed.

Sarcoid-like granulomas can be seen in PBC, chronic berylliosis, brucellosis, drug-induced injury, and in many miscellaneous conditions. Some diseases are characterized by lesions other than the granulomas that suggest the diagnosis. For example, the liver in PBC also reveals chronic cholestasis and cholangiodestructive lesions in portal areas. Drug-induced granulomas can be accompanied by hepatocellular injury or combined hepatocellular and cholestatic injury, as is typical of the liver injury associated with several drugs (23,50).



▪ **Figure 9.81** Sarcoidosis. Old, partially healed granulomas are surrounded by fibrosis and contain both epithelioid histiocytes and giant cells.

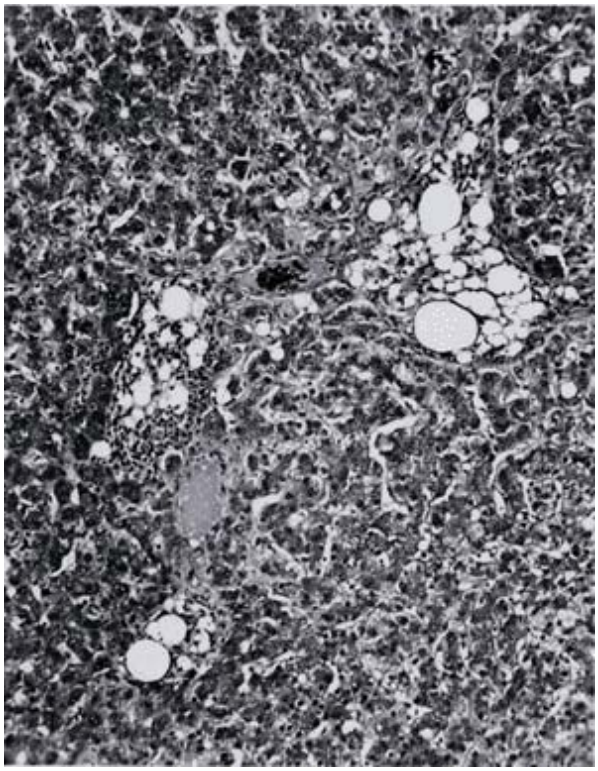
Lipogranulomas in the liver are a common finding, the result of the accumulation of ingested mineral oil (110). They consist of variable numbers of fat vacuoles, histiocytes, mononuclear cells, and sometimes eosinophils or neutrophils, and there can be some associated focal fibrosis (Fig. 9.82). Typically, lipogranulomas are located in portal areas or in the vicinity of terminal hepatic venules.

Metabolic Diseases

Identification of storage products

In the broadest sense, the term *storage* can be used to include various lesions and diseases characterized by the abnormal or excessive accumulation of a metabolite or substance in one of the cellular or extracellular compartments of the liver. This may include storage in hepatocytes, reticuloendothelial cells (Kupffer cells or other macrophages), stellate cells, and other mesenchymal cells, canaliculi, ductules, bile ducts, space of Disse, and other parts of the vasculature. Storage may indicate an inherited metabolic disease (111), or it may be

part of some other process. When an abnormal substance is noted, it can often be identified by its appearance in routine sections and by its reactions with special histochemical stains. Special techniques, such as transmission or scanning electron microscopy, fluorescence or polarizing microscopy, or immunohistochemistry, may help in select cases.



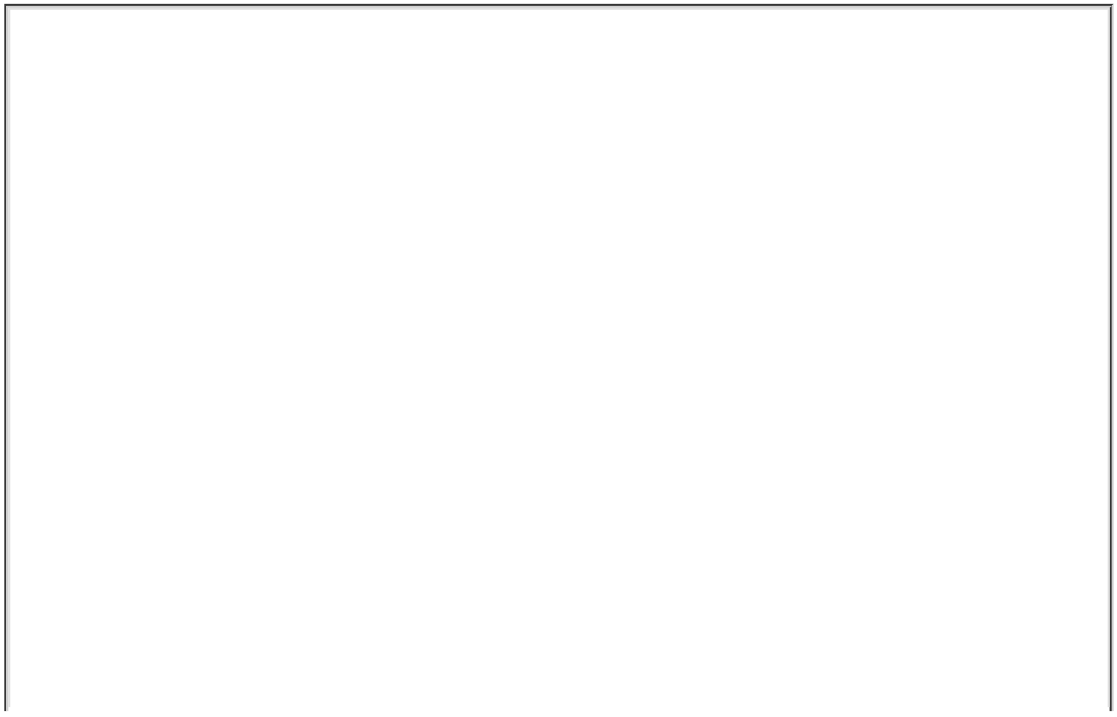
▪ **Figure 9.82** Three small lipogranulomas, composed of mineral oil droplets, macrophages, and chronic inflammatory cells.

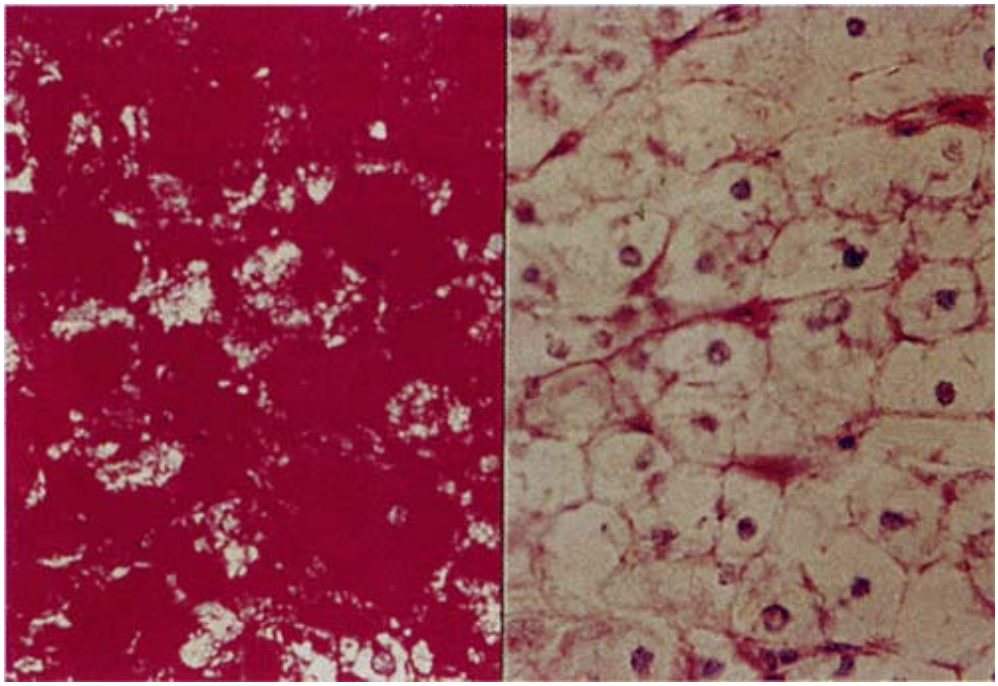
Pigment storage is one of the more common lesions. This can be recognized as brown, green, or black material, which may be stored in hepatocytes, macrophages, or canaliculi. *Bile* pigment varies from brown to green and may be found in canaliculi, ductules, or ducts in cholestatic diseases (see preceding text), which rarely presents an identification problem. Bile pigment in hepatocytes and Kupffer cells can usually be distinguished from other pigments by a characteristic green color in a Hall's stain for bile or by a greenish brown color in a Prussian blue iron stain. *Lipofuscin* pigment varies from dark brown to golden brown. It is found in hepatocytes ("wear and tear" pigment) as a normal part of aging and in increased amounts in patients with chronic drug ingestion and Dubin-Johnson syndrome; it is found in Kupffer cells as the breakdown product of phagocytosed cellular debris when there has been necroinflammatory injury or other forms of tissue necrosis. Lipofuscin stains positively with argentaffin and PAS stains (Fig. 9.4)

and negatively with the iron stain. *Hemosiderin* is brown and coarsely granular and refractile in routine stains. It accumulates in hepatocytes, Kupffer cells, and mesenchymal cells to varying

degrees in hemochromatosis and other iron overload states, and, like lipofuscin, it accumulates in Kupffer cells when there has been necroinflammatory injury. Hemosiderin stains positively with the Prussian blue stain for iron (Fig. 9.6), and so this stain can be easily used in most cases to distinguish the three brown pigments from one another.

Glycogen storage is a physiologic function of the hepatocytes. In the fed state, glycogen can be demonstrated in liver cell cytoplasm with the PAS stain, and predigestion with diastase will abolish the staining. If glycogen storage disease is suspected, a portion of the biopsy specimen should be fixed in alcohol, rather than formalin, because this is the most suitable fixative for the histochemical demonstration of glycogen. In glycogen storage diseases, the glycogen often accumulates to such an extent that the hepatocytes appear swollen and plant-like (Fig. 9.83) (111). Definitive diagnosis, however, depends on the demonstration of the specific enzymatic defect. Type IV glycogen storage disease differs from the other types and is the only type that can be readily diagnosed on liver biopsy (112). This type is associated with the accumulation of an abnormal glycogen molecule, an amylopectin-like material, in hepatocytes. This distinctive material is homogeneous and slightly eosinophilic or even colorless. It typically appears as a circumscribed inclusion (Fig. 9.84) displacing the remainder of the cytoplasm and the nucleus to the periphery and stains intensely with PAS, Best's carmine, colloidal iron, and Lugol's iodine. The inclusions resist digestion with diastase or amylase but can be digested with pectinase.

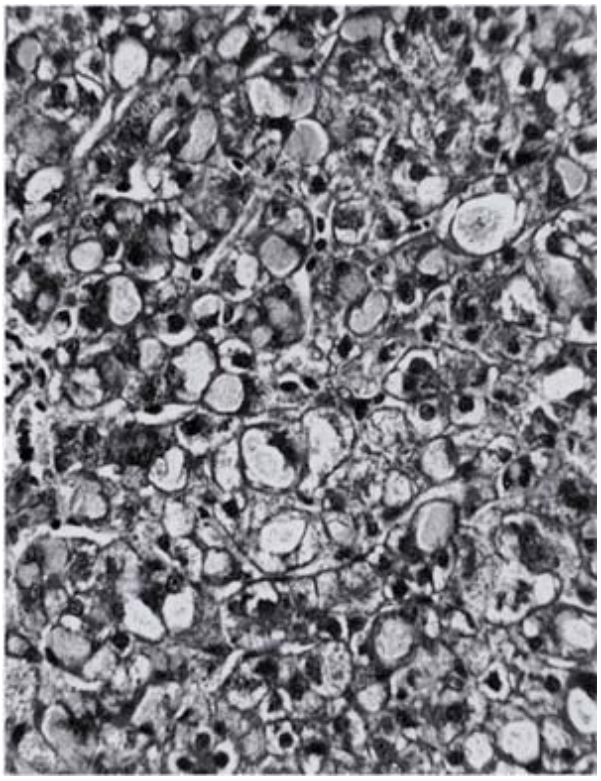




▪ **Figure 9.83** Type I glycogen storage disease. Periodic acid-Schiff stain (*left*) shows that the enlarged hepatocytes are filled with glycogen, which is removed by diastase digestion (*right*), showing the liver cells to have a clear, finely vesiculated cytoplasm.

Proteins and glycoproteins are stored in hepatocytes in several conditions. Proteins stain with eosin in routine hematoxylin and eosin sections, so they can be recognized as storage material when they form discrete cytoplasmic inclusions. *a_1-Antitrypsin (AAT) deficiency* (see Chapter 37) is the best-characterized disorder with protein storage. AAT, a major protease inhibitor (Pi) in serum, is a glycoprotein that is synthesized predominantly by the liver to function as a modulator of the inflammatory response by inhibiting proteases. Individuals with one or both AAT alleles of the "Z" phenotype are unable to transport and secrete newly synthesized AAT molecules normally through the endoplasmic reticulum and Golgi apparatus of the hepatocyte, resulting in low concentrations of AAT in the serum. Faulty secretion of abnormal AAT molecules coupled with defective degradation of these molecules retained in the Golgi apparatus leads to the formation of characteristic eosinophilic globules in hepatocytes (Fig. 9.85) (113). The globules are PAS positive and diastase resistant (Fig. 9.5) due to the carbohydrate moieties of the glycoprotein. These are typically found in patients who are homozygous or heterozygous for PiZ, but they can also occur in other phenotypes, even sometimes in patients with the normal PiM phenotype (114,115). In the noncirrhotic liver, the characteristic AAT globules are located in periportal hepatocytes.

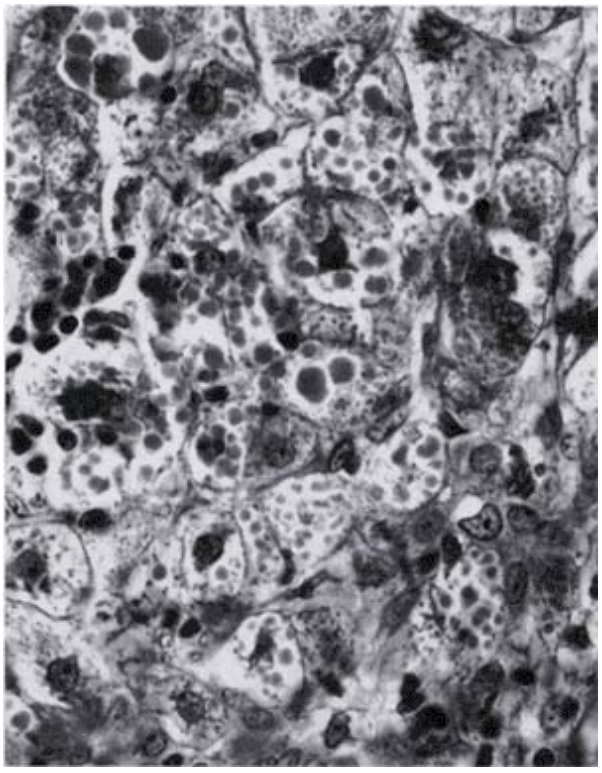
They are round, homogeneous, and eosinophilic, and vary from 1 to 40 μm in diameter. Usually, they are separated from the remainder of the cytoplasm by a halo that is probably artifactual. Immunohistochemical staining can confirm that the globules are composed of AAT but cannot be used to determine the phenotype. PAS-positive globules are usually not detectable in infants younger than 3 months, although some present with neonatal hepatitis or paucity of bile ducts. Liver biopsy specimens from adults with AAT deficiency may reveal only the characteristic globules in periportal regions, or there may be erosion of the limiting plate associated with chronic inflammation and periportal fibrosis, similar to chronic hepatitis of other etiologies, or cirrhosis with globules at the periphery of the nodules.



▪ **Figure 9.84** Type IV glycogen storage disease. The abnormal glycogen metabolite accumulates in cytoplasmic inclusions.

Other protein storage disorders, both inherited and acquired, may produce cytoplasmic inclusions. α_1-Antichymotrypsin deficiency and antithrombin III deficiency are rare, but both are associated with AAT-like PAS-positive globules (116,117). Familial fibrinogen storage disease produces globular inclusions that are eosinophilic but only weakly PAS-positive because of a lower carbohydrate content (118). Plasma protein inclusions (119), consisting of a mixture of circulating proteins imbibed

by hepatocytes from the plasma, are seen most often in hepatic congestion. They are variably PAS positive and may be globular or have a pale eosinophilic appearance that can be confused with the ground-glass inclusions of hepatitis B. PAS-positive, diastase-resistant, ground-glass–appearing inclusions are also seen in patients taking the drug cyanamide (120) (not used in the United States) and in patients with Lafora's disease (myoclonus epilepsy) (121).

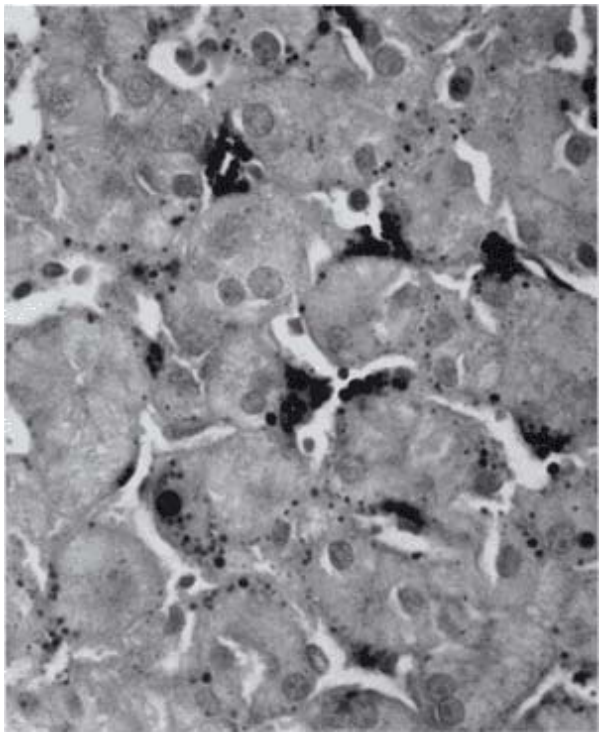


▪ **Figure 9.85** α_1-Antitrypsin deficiency. The hepatocytes contain eosinophilic globules of α_1-antitrypsin stored in endoplasmic reticulum cisternae.

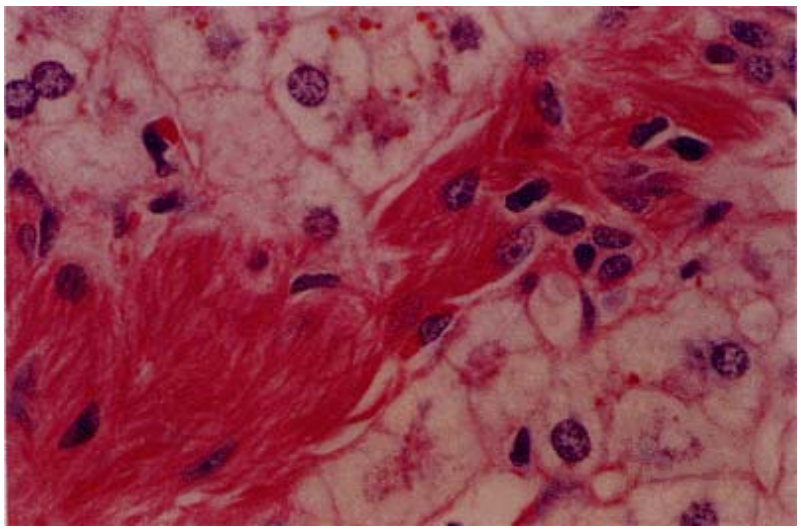
Lipids, glycolipids, sphingolipids, and other phospholipids accumulate in a number of inherited and acquired conditions. Hepatocytes, Kupffer cells, and stellate cells that contain a lipid storage product generally appear clear, vacuolated, or foamy. Triglyceride is by far the most common storage product, and this is discussed separately in the preceding text under Steatosis. All lipids stain positively in unprocessed frozen sections with the oil red O and Sudan black stains. Most, however, do not survive routine processing in organic solvents, so their demonstration requires forethought because a portion of the specimen must be handled separately. To avoid the artifacts of frozen section, the tissue can be postfixed in osmium tetroxide, which stains the lipid black (Fig. 9.86) (122). Cholesterol esters are stored along with triglyceride

in the two forms of lysosomal acid lipase deficiency, Wolman's disease and cholesterol ester storage disease, causing the hepatocytes to appear swollen and pale. Both types of lipid stain with oil red O, but the cholesterol can also be demonstrated in frozen

sections with a Schultz stain (1). Glucosylceramide, a glycolipid, accumulates in Kupffer cells in Gaucher's disease (lysosomal glucocerebrosidase deficiency), producing the distinctive striated appearance of Gaucher's cells. The PAS stain after diastase digestion provides an excellent demonstration of these cells because enough of the carbohydrate component of the storage product survives processing and stains positively (Fig. 9.87). Sphingomyelin accumulates in Kupffer cells in the many variants of Niemann-Pick disease, producing a foamy appearance (Fig. 9.88). Other phospholipids accumulate in both Kupffer cells and hepatocytes in drug-induced phospholipidosis because of chronic ingestion of amphophilic drugs such as amiodarone (50). The Kupffer cells appear foamy, but the hepatocellular phospholipid storage can generally only be recognized by electron microscopy (Fig. 9.89). Vitamin A is stored in stellate cells (formerly called *perisinusoidal lipocytes or Ito cells*), and these become prominent in individuals ingesting excess vitamin A (Fig. 9.90). Lipid globules containing the vitamin A are apparent with fat stains, and vitamin A is autofluorescent in frozen sections. Mineral oils (paraffins) are common in the Western diet, and some are absorbed and stored in portal macrophages or lipogranulomas near terminal hepatic venules (Fig. 9.82). In frozen sections the mineral oil stains a pale salmon with the oil red O stain.



▪ **Figure 9.86** Osmium stain for fat. Lipids stain black when the tissue is postfixed in osmium tetroxide. In this patient with hypervitaminosis A, the stellate cells (perisinusoidal lipocytes) are hypertrophied because of vitamin A storage, and there are a few small triglyceride droplets in hepatocytes.



▪ **Figure 9.87** Gaucher's disease. Striations in the Gaucher's cells are nicely demonstrated by the PAS stain after diastase digestion.

Mucopolysaccharides accumulate in both hepatocytes and Kupffer cells in Hunter and Hurler diseases (Fig. 9.91), other mucopolysaccharidoses, and mucolipidoses. The affected cells appear swollen and finely vacuolated. Colloidal iron, alcian blue, and other stains for mucopolysaccharides are positive, and the PAS stain after diastase digestion is also positive. Oligosaccharides in diseases such as sialidosis are stored in hepatocyte lysosomes, making the liver cells appear vacuolated.

Porphyrins accumulate in the liver in porphyria cutanea tarda and erythropoietic protoporphyria (see Chapter 38). Uroporphyrin crystals in hepatocytes are

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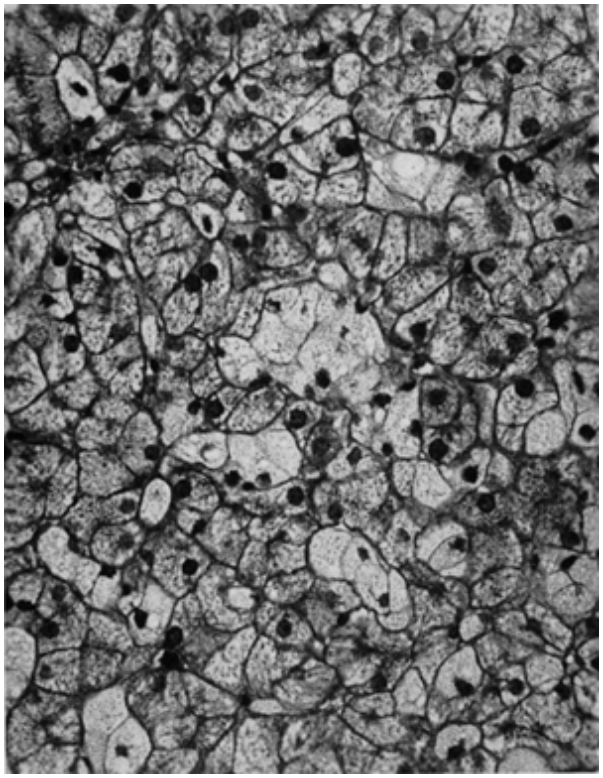
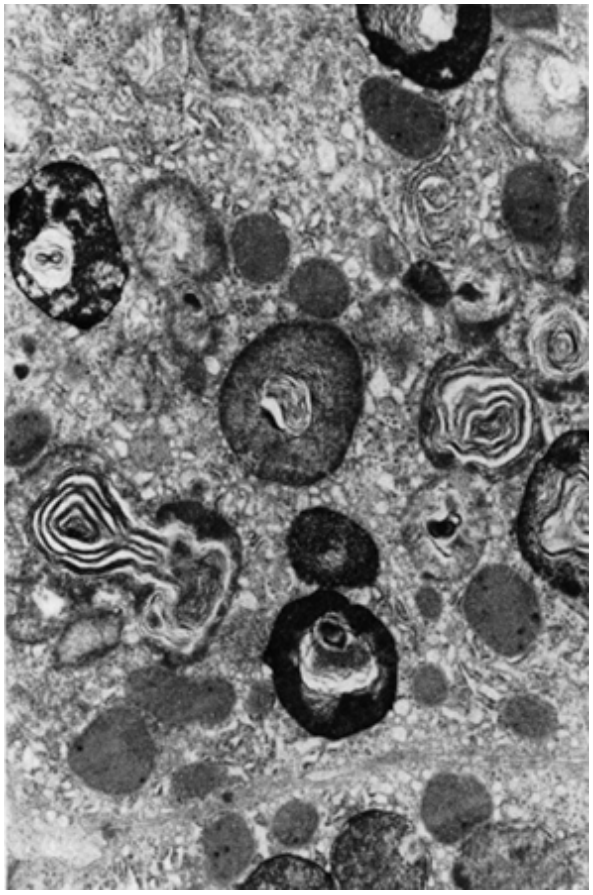
inapparent by routine stains, but these can sometimes be demonstrated with a ferric ferricyanide stain (123) in patients with porphyria cutanea tarda. Protoporphyrin deposits in erythropoietic protoporphyria appear as brownish red globular masses in hepatocytes and bile canaliculi. They are easily mistaken for bile pigment, but with polarized light they

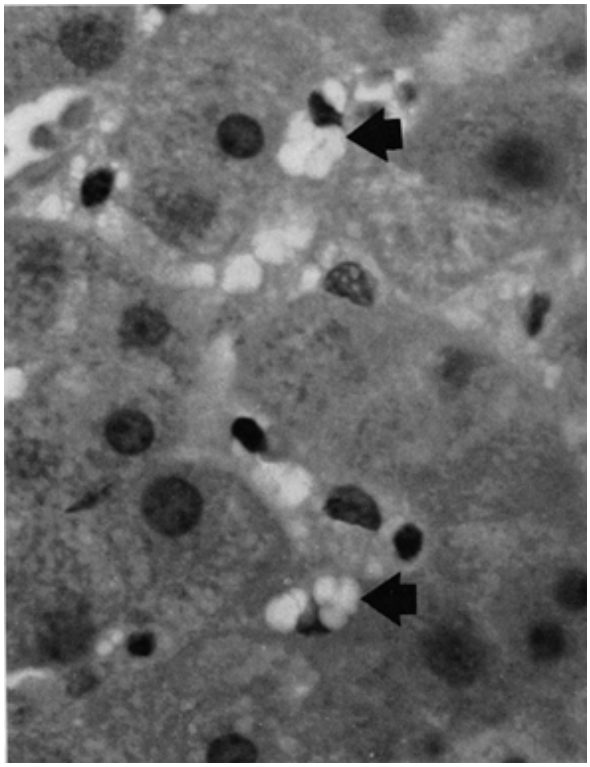
are red with characteristic Maltese cross birefringence (Fig. 9.15) (13).



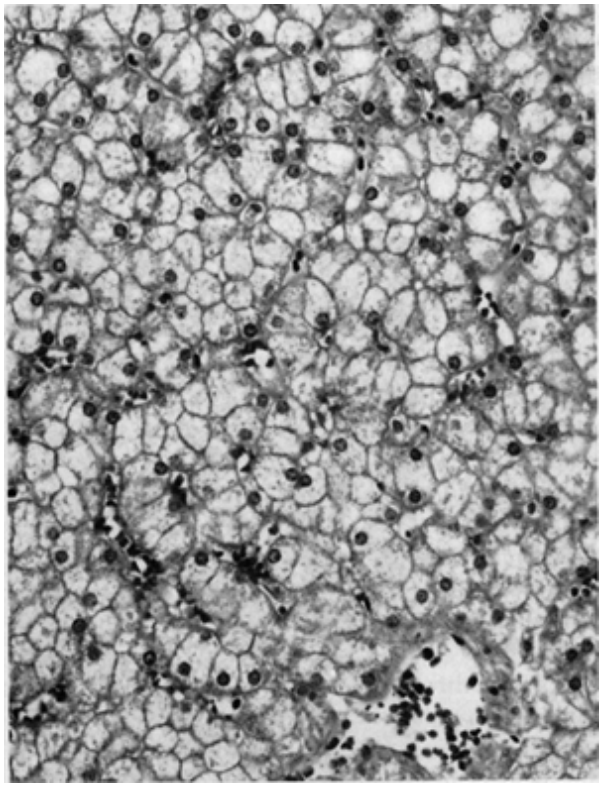
▪ **Figure 9.88** Niemann-Pick disease. Sphingomyelin accumulates in Kupffer cells, giving them a foamy appearance. Liver cells appear normal.



▪ **Figure 9.89** Phospholipidosis in a patient taking amiodarone. Ultrastructurally the hepatocyte cytoplasm contains numerous lamellated whorls of phospholipid.



▪ **Figure 9.90** Hypervitaminosis A. Stellate cells (*arrows*) are engorged with stored vitamin A.



▪ **Figure 9.91** Hurler disease. The stored mucopolysaccharide gives the swollen liver cells a finely vacuolated appearance.

Copper storage in hepatocytes in chronic cholestatic diseases or in Wilson disease is not visible by light microscopy, but it is often found in association with periportal or periseptal lipofuscin granules. Stains for copper, such as rhodanine (Fig. 9.7), are needed for demonstrating storage because it can easily be missed.

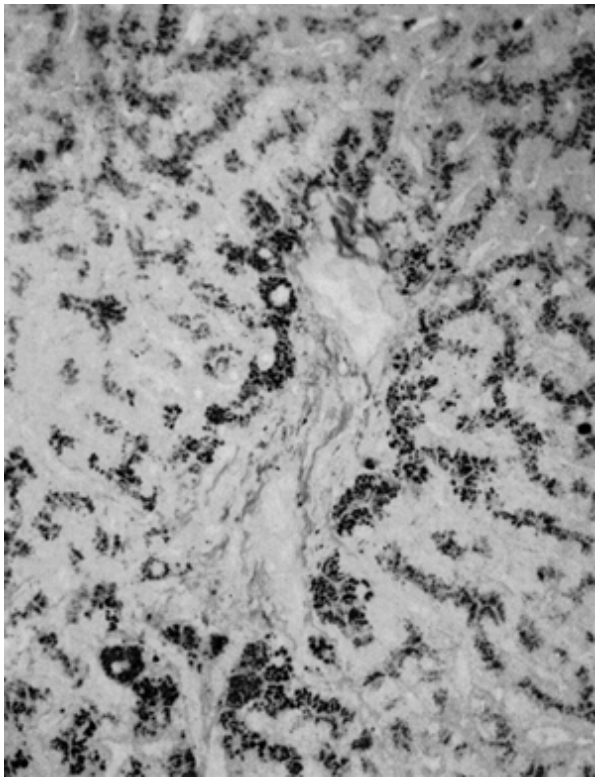
Crystals of various types may be stored in macrophages under special conditions. Many crystals are birefringent with polarized light, and scanning electron microscopy with x-ray spectrophotometry can specifically identify those that are inorganic. In cystinosis, crystals of cystine can be demonstrated with the polarizer in the tissue that has been fixed in alcohol. Birefringent talc crystals (Fig. 9.14) or black, nonbirefringent titanium dioxide pigment granules can sometimes be found in macrophages of some patients who are or had been intravenous drug abusers. Other foreign materials, such as gold (which appears as a black pigment in patients treated with gold for rheumatoid arthritis), thorium dioxide (Thorotrast, a discontinued radiologic contrast material), polyvinyl pyrrolidone (a discontinued plasma expander), and anthracotic carbon (in coal miners), may be found stored in reticuloendothelial cells.

Genetic hemochromatosis

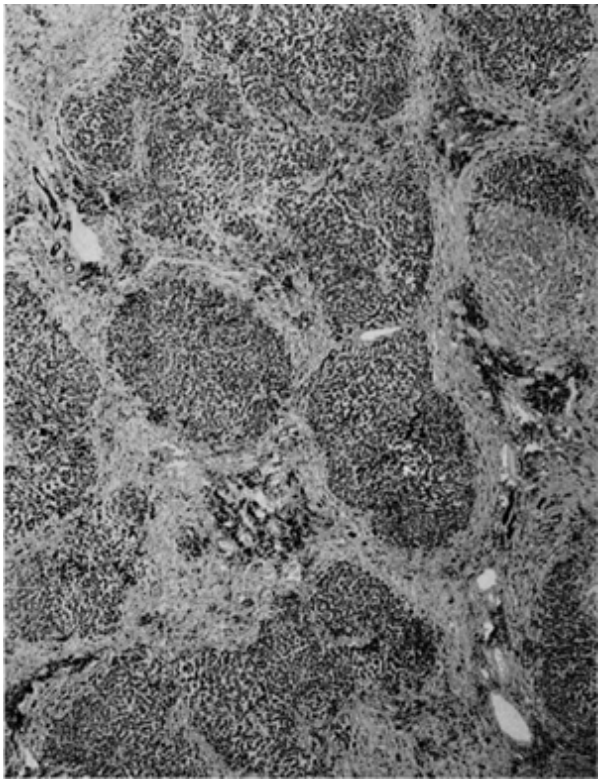
As the most common hepatic storage disease, biopsy diagnosis of hemochromatosis deserves special mention. Gene analysis and quantization of iron concentration in liver tissue obtained by biopsy are now the preferred method for diagnosis (see Chapter 36), but examination of the biopsy specimen is still useful in distinguishing primary from secondary iron overload and in assessing the degree of fibrosis. *Secondary iron overload* is the proper term for nongenetic causes of excess tissue iron. This may be due to multiple blood transfusions, chronic hemolysis, or prolonged dietary overload, but it rarely results in tissue damage except in a few extreme instances. *Hemosiderosis* is the term used for morphologically identifiable iron accumulation in tissue, whatever the cause. Severe hemosiderosis is usually due to genetic hemochromatosis, but it can be secondary to transfusional or dietary iron or chronic hemolysis. In such cases the hemosiderin accumulation is predominantly in reticuloendothelial cells. Excess iron may accumulate in hepatocytes of patients with damaged livers, especially in alcoholic cirrhosis and also in chronic viral hepatitis. Many of these patients are probably heterozygous for genetic hemochromatosis.

In homozygous genetic hemochromatosis, iron accumulates over the course of the patient's life (124). In young homozygous patients, this is detected as a progressive increase in hepatocellular hemosiderin pigment (most prominently in periportal regions) with minimal or no other pathologic changes (Fig. 9.92). As the quantity of iron increases, the cells of zones 2 and 3 become affected. Scattered apoptotic bodies and foci of necrosis are found infrequently. Kupffer cells may ingest small quantities of iron. By middle age in men or after menopause in women, enough iron has usually accumulated to cause hepatocellular necrosis, portal inflammation, and portal and bridging fibrosis. Alcohol and intercurrent liver diseases such as hepatitis C may accelerate iron accumulation. Fibrous septa eventually creep from the portal areas into the surrounding parenchyma. Evidence of regeneration is not apparent in the precirrhotic stage, but a reticulin stain can demonstrate plates greater than one cell in thickness near the portal tracts. The fibrous septa can

show variable ductular proliferation, but inflammation is mild or absent.



▪ **Figure 9.92** Hemochromatosis. This iron stain of a biopsy from a young homozygous patient shows a marked increase in hepatocyte stainable iron (dark granules), most prominently in acinar zone 1. There is no fibrosis as yet.



▪ **Figure 9.93** Micronodular cirrhosis of hemochromatosis. The iron stain shows marked deposition of hemosiderin (dark granules) in liver cells, bile ducts, and mesenchymal cells of the fibrous septa.

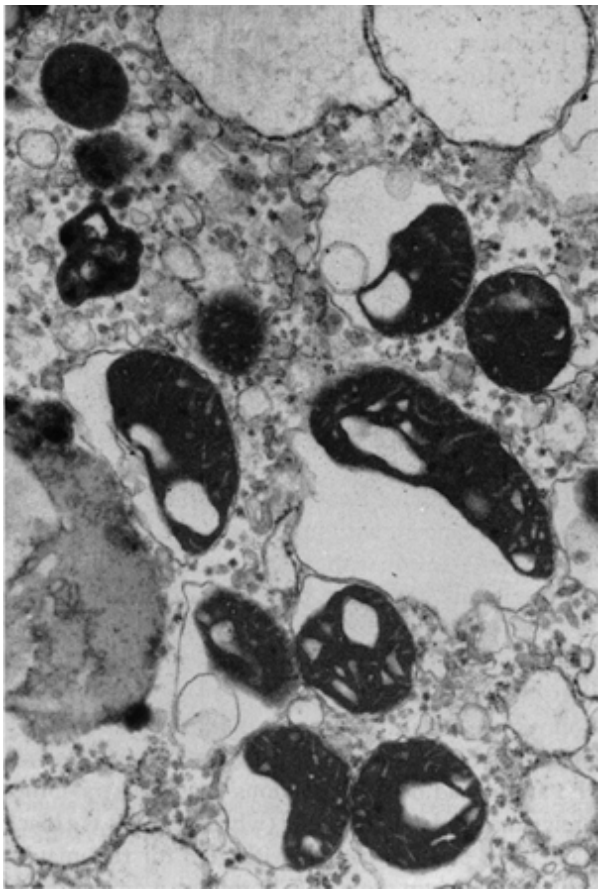
Fibrous bands from adjacent portal tracts eventually join and dissect the parenchyma into irregular micronodules (Fig. 9.93). By this stage there is marked hemosiderin deposition with heavy pigment staining of hepatocytes, bile duct epithelium, ductules, and mesenchymal cells of the fibrous septa and vessels. Relatively less iron is found in Kupffer cells. Regeneration is not usually prominent, but regenerative nodules offer striking contrast to the remaining parenchyma by their lack of stainable iron, so-called iron-free foci (125). Hepatocellular carcinomas that develop in hemochromatotic livers are also devoid of iron, and there is evidence that the iron-free foci represent preneoplastic lesions.

Wilson disease

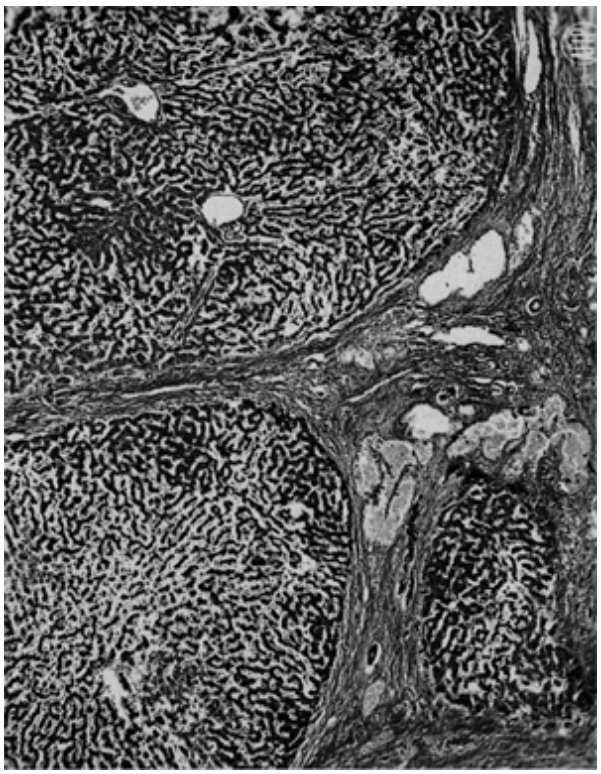
Tissue damage in Wilson disease (see Chapter 35) is related to excess copper, and the liver is the earliest site of progressive copper accumulation, after which the copper is released into the blood to accumulate in other organs. The histopathologic changes (126,127,128) are not specific and must be evaluated in conjunction with clinical and laboratory findings. Hepatic copper concentration can be measured in the tissue obtained by needle biopsy and can provide a definitive

diagnosis when other tests are equivocal. Biopsy specimens obtained from young siblings of patients with this disease may show little or no hepatic damage. The earliest microscopic lesions include steatosis, periportal glycogenated nuclei, and rare foci of necrosis or apoptotic bodies. Although the hepatic copper content is elevated, copper is usually not histochemically identifiable at this stage, but ultrastructural changes in hepatic mitochondria, thought to be characteristic if not pathognomonic, are present (Fig. 9.94). These include pleomorphism, separation of the inner and outer membranes, enlarged intercristal spaces, and various types of inclusions (6,129). More advanced cases show lesions that resemble chronic hepatitis of other cause. Mallory bodies, with their characteristic neutrophilic response, may be present in the liver cells in zone 1. Copper may be demonstrable in the periportal areas with appropriate stains. A variety of patterns of cirrhosis can develop in the later stages but a macronodular type is the most common (Fig. 9.95). Regenerative foci lack identifiable copper, so absence

of stainable copper in a cirrhotic biopsy, particularly if the sample is small, does not rule out Wilson disease. On the other hand, a large amount of copper in a cirrhotic liver is strongly suggestive of Wilson disease (Fig. 9.7).



▪ **Figure 9.94** Wilson disease. Ultrastructurally, the mitochondria have a dense matrix with separation of the membranes of the cristae.



▪ **Figure 9.95** Wilson disease, end stage. Macronodular cirrhosis is present with large nodules separated by bands of scar tissue.

Vascular Disorders

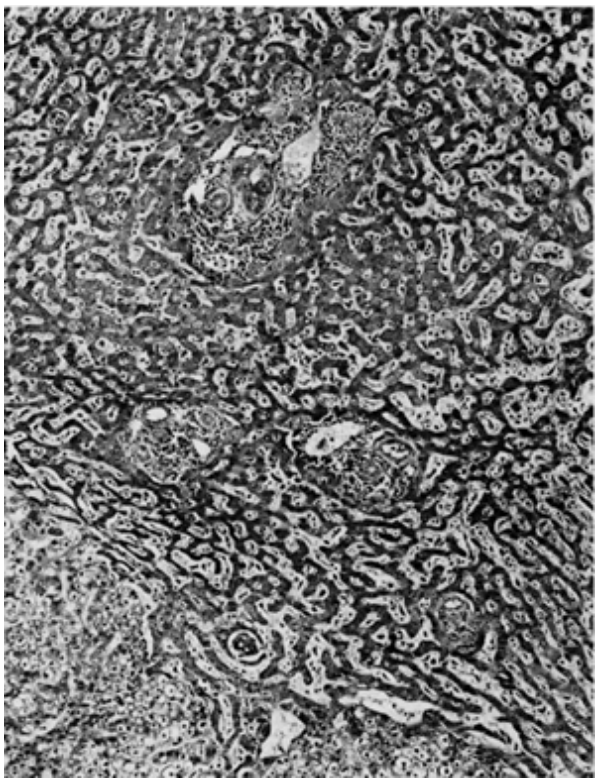
Vascular patterns of injury

Certain patterns of injury in the liver are typical, if not pathognomonic, of a vascular disease. Congestion, atrophy, and coagulative necrosis are all findings that suggest a vascular component to the underlying disorder. A variety of substances, both endogenous and foreign, can be deposited in the vasculature as well.

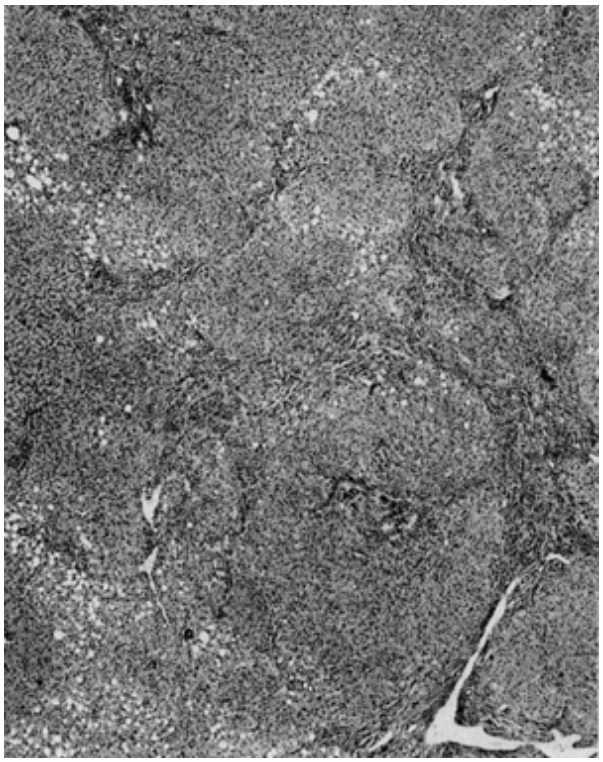
Ischemia. Acute ischemic injury typically produces zone 3 ("centrilobular") coagulative necrosis, similar to that seen in severe injury from certain toxins, such as acetaminophen overdose or mushroom poisoning. Ischemic injury may follow shock, left-sided heart

failure, or right-sided failure associated with hypotension (130,131). Clinically, the presentation may mimic viral hepatitis (ischemic hepatitis) (132). Liver cells that have undergone coagulative degeneration are shrunken, have an intensely eosinophilic cytoplasm, and show nuclear pyknosis or lysis (Fig. 9.20). When an inflammatory response is present, it is invariably neutrophilic. Kupffer cells are hypertrophied and usually full of lipofuscin. Clearing of the dead hepatocytes leads to condensation of reticulin fibers and fibrosis in some cases.

Atrophy. Chronic ischemic injury produces atrophy of acini or liver cell plates. Mild forms of chronic ischemia are a fairly common consequence of aging. Mild portal fibrosis with some periductal fibrosis and reduction in portal vein diameter is a frequent finding in elderly individuals. Often there will be areas in the liver in which vascular structures appear close together, indicating that the acini have atrophied (Fig. 9.96). Extreme forms of this phenomenon can result in hepatoportal sclerosis (also called *idiopathic portal hypertension, noncirrhotic portal hypertension, or noncirrhotic portal fibrosis*) (133) or nodular regenerative hyperplasia (134) when there is heterogeneous blood flow through portal vein branches and hepatic arterioles with atrophy of the affected acini and compensatory hyperplasia of other acini (Fig. 9.97). Both conditions may cause portal hypertension in the absence of cirrhosis. Atrophy of the left lobe and segmental atrophy (Fig. 9.98) (135) are usually due to severe compromise of the vasculature (inflow or outflow) to a portion of the liver, but this may also be due to bile duct occlusion.



▪ **Figure 9.96** Atrophy, secondary to chronic ischemia. Portal areas are fibrotic and close together, indicating atrophy of the acini.



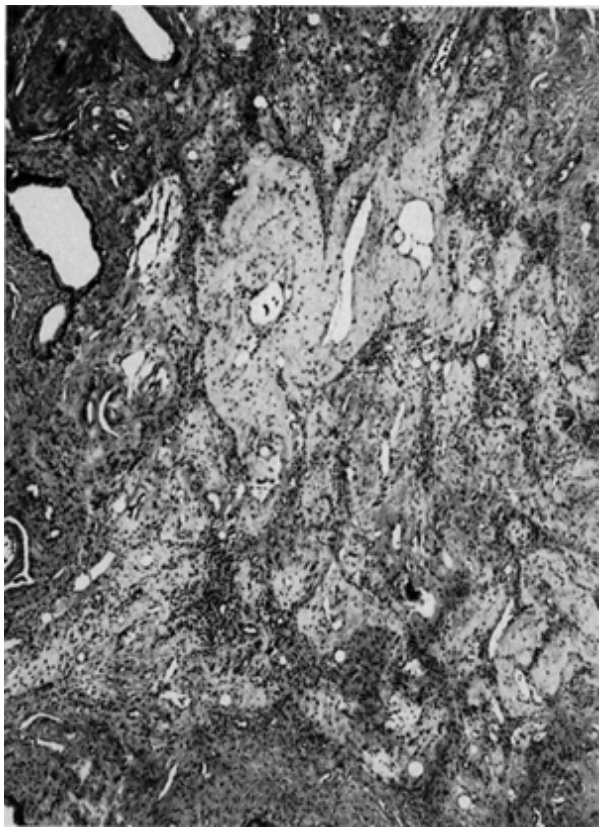
▪ **Figure 9.97** Nodular regenerative hyperplasia. The entire hepatic parenchyma is replaced by hyperplastic nodules separated by atrophic liver cell plates.

Venous outflow obstruction

The terminal hepatic venules ("central veins"), intercalated ("sublobular") veins, hepatic veins, and inferior vena cava form the venous outflow tract, but only the terminal hepatic venules and intercalated veins are commonly sampled by liver biopsy. Obstruction of any portion of the outflow tract can be followed by changes in other vessels, such as sinusoidal dilatation and, rarely, thrombosis of portal venules. Chronic congestive heart failure or constrictive pericarditis can mimic outflow tract obstruction when severe.

Hepatic vein thrombosis (Budd-Chiari syndrome) produces histologic findings that are commonly confused with congestive heart failure or drug-induced injury and must be interpreted with care. Many of the

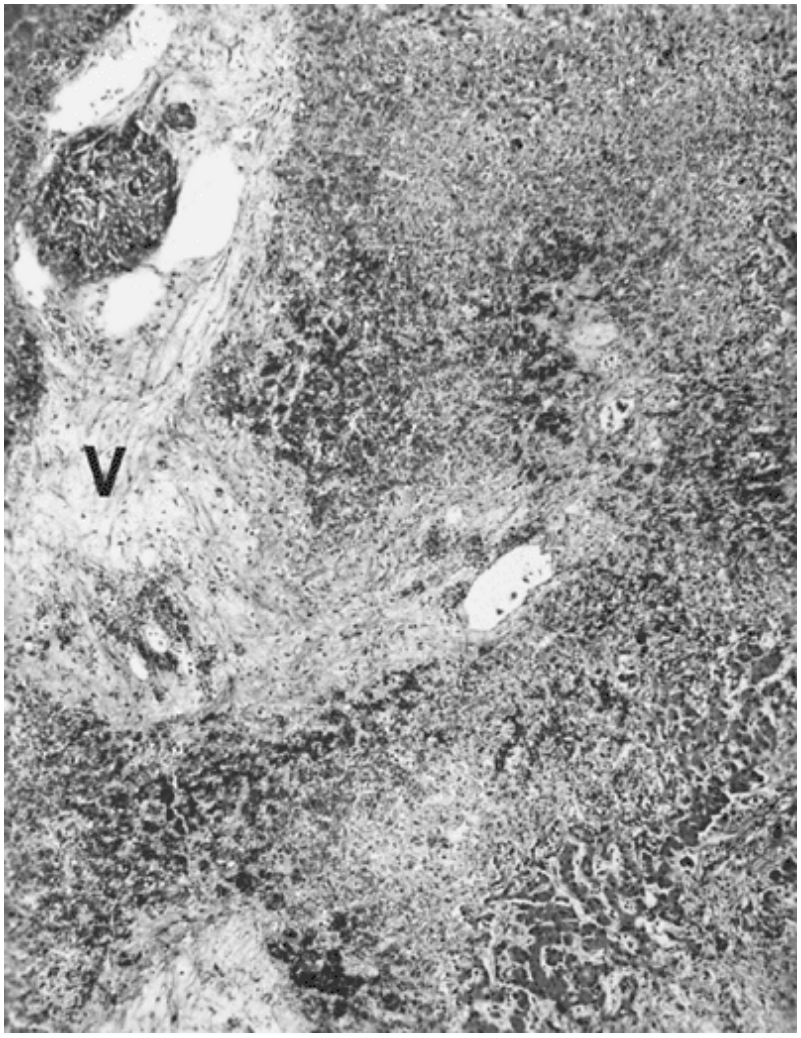
changes do resemble those of congestive heart failure, but differ by showing variability of involvement among acini, particularly well visualized with open (wedge) biopsy specimens. Those acini with acute changes show severe sinusoidal dilatation and congestion, most pronounced in zone 3 (Fig. 9.99), but sometimes extending to the portal tracts. Coagulative degeneration or necrosis is frequently present. Erythrocytes in the congested areas are packed into the spaces of Disse and crowd the degenerating hepatocytes. The terminal hepatic venules and intercalated veins can show thrombosis, recanalized thrombi (Fig. 9.99), and/or fibrous mural thickening, but in a small biopsy specimen the veins that are sampled may be normal. Some acini are injured in a more gradual manner. Progressive sinusoidal dilatation is accompanied by atrophy of hepatocytes. Zone 3 fibrosis can follow either type of injury and can link adjacent terminal hepatic venules. Small portal veins may also become occluded, further augmenting the injury to parts of the liver (136). Other acini are entirely spared. The caudate lobe is often uninvolved because of its separate venous drainage and can undergo compensatory hypertrophy.



▪ **Figure 9.98** Segmental atrophy. The tissue is fibrotic, and there are portal areas and ductules, but hepatocytes are missing, presumably because of chronic ischemia.

Veno-occlusive disease resulting from toxic injury (e.g., from pyrrolizidine alkaloids) or radiation damage to the endothelium of the small outflow produces parenchymal changes that resemble those of Budd-Chiari syndrome, but in the early stages, lesions of the terminal hepatic venules and intercalated veins are distinctive. Intimal edema is followed by the subendothelial deposition of reticulin and collagen fibers and progressive narrowing of the lumen (Fig. 9.100). Extravasated erythrocytes are frequently

situated between the fibers. Inflammation is sparse or absent, and superimposed thrombi are not seen. Cases with severe acute injury may show necrosis of venous walls. With progressive fibrosis of the walls, the veins become difficult to identify, appearing as small hyalinized cylinders. Involvement of hepatic veins or the vena cava does not occur in most cases.

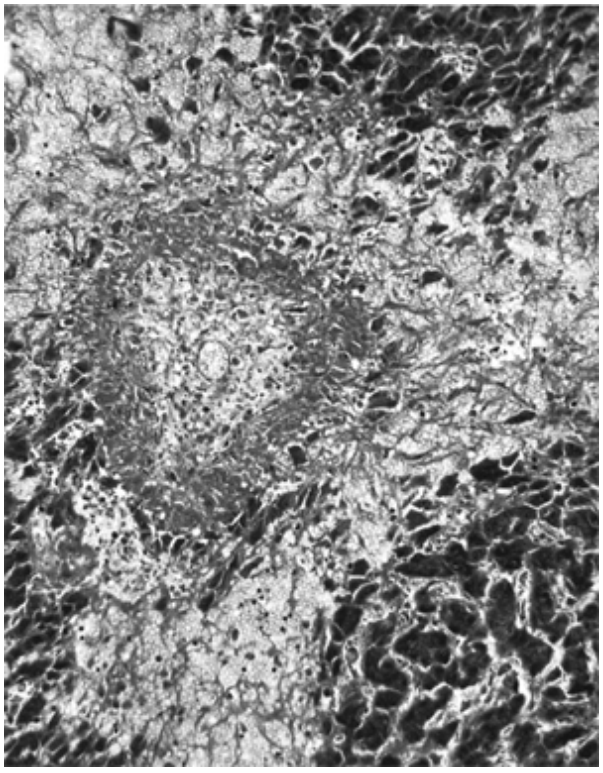


▪ **Figure 9.99** Budd-Chiari syndrome. Organizing thrombus in a large intercalated vein (*V*) is associated with severe sinusoidal dilatation and congestion and necrosis of zone 3 of the surrounding

acini.

Sinusoidal lesions

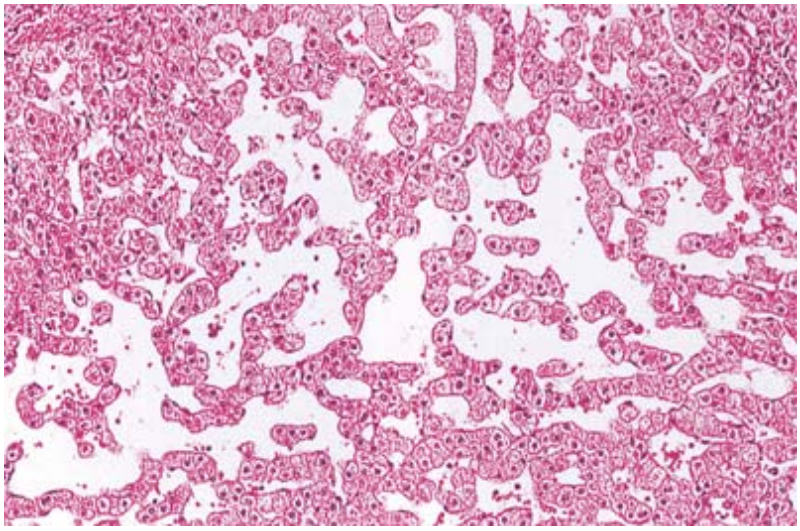
Dilatation of sinusoids is a frequent finding in liver biopsy specimen and is often a nonspecific reaction to systemic disease. Chronic congestive heart failure leads to the gradual development of dilatation and congestion of sinusoids and finally to atrophy of hepatocytes, predominantly in zone 3, with secondary fibrosis (130,131). A characteristic type of periportal (zone 1) sinusoidal dilatation sometimes follows the use of oral contraceptives (137). Liver plates show variable degrees of atrophy (Fig. 9.101). The change affects all acini, unlike the focal sinusoidal dilatation seen sometimes near hepatic masses. Panacinar sinusoidal dilatation can be found in sickle cell disease; the dilated sinusoids are packed with masses of sickled erythrocytes. Variable dilatation, lacking any particular zonal localization, can be associated with other disorders, notably neoplasms and granulomatous diseases. Sinusoidal dilatation of the liver may be a systemic manifestation of a number of neoplasms. A characteristic triad of histologic changes, consisting of focal sinusoidal dilatation, proliferation of ductules, and infiltration of edematous portal areas by neutrophils, has been observed in the vicinity of space-occupying lesions (138).



▪ **Figure 9.100** Veno-occlusive disease associated with Senecio alkaloid toxicity. The efferent vein has a markedly narrowed lumen because of intimal thickening with extravasation of erythrocytes. There is zone 3 necrosis and marked congestion.

Peliosis hepatis is characterized by scattered lakes of blood of varying sizes, which appear to represent an extreme degree of localized sinusoidal dilatation (Fig. 9.102). The pathogenesis of the process is now considered to be due to endothelial injury that allows blood to accumulate in spaces of Disse with resultant formation of the cavities. In the past, peliosis was recognized as a complication of debilitating disorders, such as tuberculosis and malignancies, and was discovered at autopsy as an incidental finding. Currently, the most frequent cause is therapy with androgenic/anabolic steroids (139). Histologically, the peliotic lakes may have an attenuated endothelial lining. Varying degrees of sinusoidal dilatation can be present near some of the lesions, but the term *peliosis hepatis* should not be used for simple sinusoidal dilatation. Bacillary angiomatosis due to *Bartonella henselae* in patients with AIDS (140)

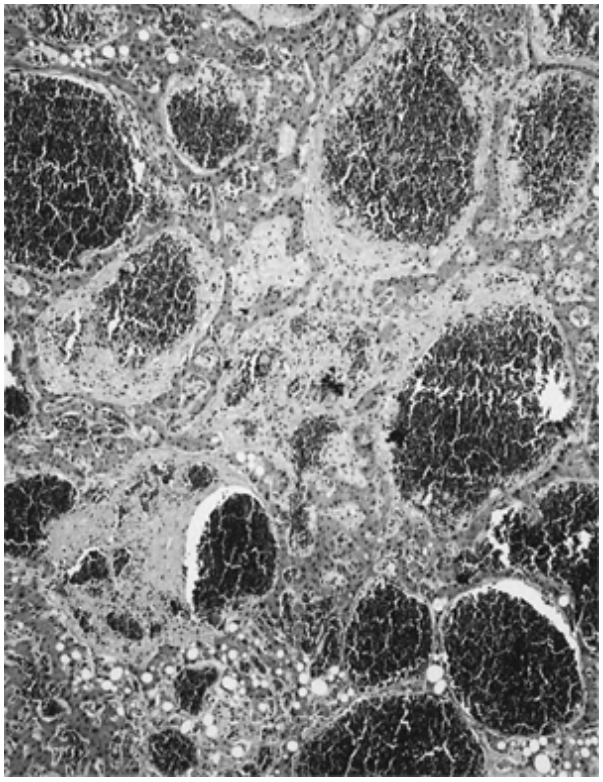
can mimic peliosis, but stains for bacteria typically demonstrate numerous organisms.



▪ **Figure 9.101** Marked periportal sinusoidal dilatation associated with long-term oral contraceptive steroid use. The liver cell plates are atrophic.

Thrombosis of sinusoids with deposition of fibrin thrombi is unusual, but

it happens in some diseases. Patients with toxemia of pregnancy usually have no evidence of liver disease; those who do frequently have deposits of fibrin in periportal sinusoids (141). Occasionally, the deposits are associated with coagulative-type hepatocellular necrosis. Disseminated intravascular coagulation can be associated with a similar pattern of injury, but the sinusoidal fibrin deposition need not be restricted to the periportal areas.



▪ **Figure 9.102** Peliosis hepatis associated with anabolic steroid therapy. Variable-sized lakes of blood are scattered throughout the parenchyma.

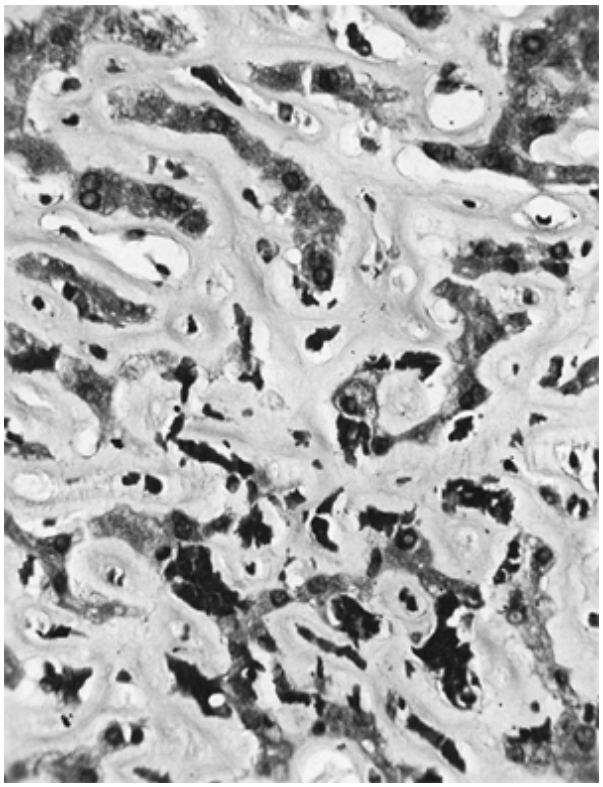
Fibrosis of sinusoids occurs in many chronic diseases. Collagen is deposited in the space of Disse along with laminin, forming basement membranes and leading to capillarization of the sinusoids. The collagen is well demonstrated with a Masson stain, and many components of basement membranes and extracellular matrix can be demonstrated with specific immunostains. Sinusoidal fibrosis is prominent in zone 3 of the acinus in the early stages of alcoholic hepatitis (Fig. 9.69), nonalcoholic steatohepatitis, diabetes mellitus, chronic congestive heart failure, and vitamin A hepatotoxicity.

Amyloidosis

Patients with amyloidosis frequently have hepatic involvement, and liver

biopsy has been advocated as a means of establishing the diagnosis. Amyloid can be limited to the arteries but can also be found in the parenchyma (Fig. 9.103). In both primary amyloidosis (type AL) and secondary amyloidosis (type AA), the eosinophilic material gradually accumulates in the space of Disse (142), eventually leading to atrophy of the hepatic plates. The presence of amyloid can be confirmed by an appropriate stain, either histochemical (e.g., Congo red with apple green dichroism

under polarized light) or immunohistochemical, with a specific antibody to the amyloid.

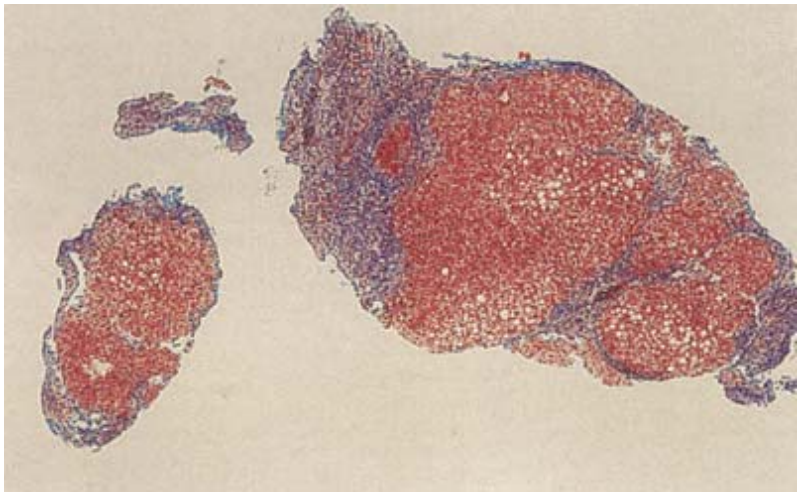


• **Figure 9.103** Extensive intra-acinar amyloid deposition filling the space of Disse between the hepatocytes and endothelial cells, producing atrophy of the liver cell plates.

Cirrhosis

Cirrhosis is defined as a diffuse process characterized by fibrosis and conversion of the normal liver architecture into structurally abnormal nodules (143). Three basic morphologic categories are recognized on the basis of the size of the cirrhotic nodules. The micronodular type includes those cases in which almost all nodules are less than 3 mm in diameter. In the macronodular type, most nodules are greater than 3

mm in diameter and usually show striking variation in size. The mixed pattern is characterized by approximately equal numbers of micro- and macronodules. Regenerative nodules are not essential for the diagnosis of cirrhosis; in both biliary cirrhosis and hemochromatosis, for example, regeneration may be minimal or absent.



▪ **Figure 9.104** Fragmented needle biopsy specimen from a cirrhotic liver. The Masson stain shows collagen of the fibrous septa enveloping and traversing the fragments.

The diagnosis of cirrhosis may be difficult to establish by percutaneous needle biopsy, particularly if the pattern is macronodular. Cutting needles (e.g., Tru-cut) are preferred because these obtain specimens that include the fibrous septa and the parenchymal nodules (Fig. 9.104). Suction techniques (e.g., Menghini needles) are limited by preferential sampling of parenchyma as the biopsy needle rebounds from the fibrous septa. There are, however, a number of microscopic clues to the diagnosis, even in this type of specimen. Suction biopsies from cirrhotic livers are commonly fragmented, and the fragments have rounded edges Fibrous septa can course through the fragments, but these are sometimes represented by thin strips hugging margins of the fragments. Stains for collagen (Fig. 9.104) are frequently necessary to detect them. Such stains are also valuable for distinguishing collapsed reticulin that follows extensive necrosis from fibrosis and for demonstrating thick liver plates in regenerative nodules of the cirrhotic liver. Reticulin stains usually demonstrate a very irregular pattern because of alterations in the growth of hepatocytes. Many cell plates are greater than one cell in thickness,

and the compressed sinusoidal spaces may be nearly invisible. Hepatocytes are pleomorphic, unless the process is entirely inactive. An

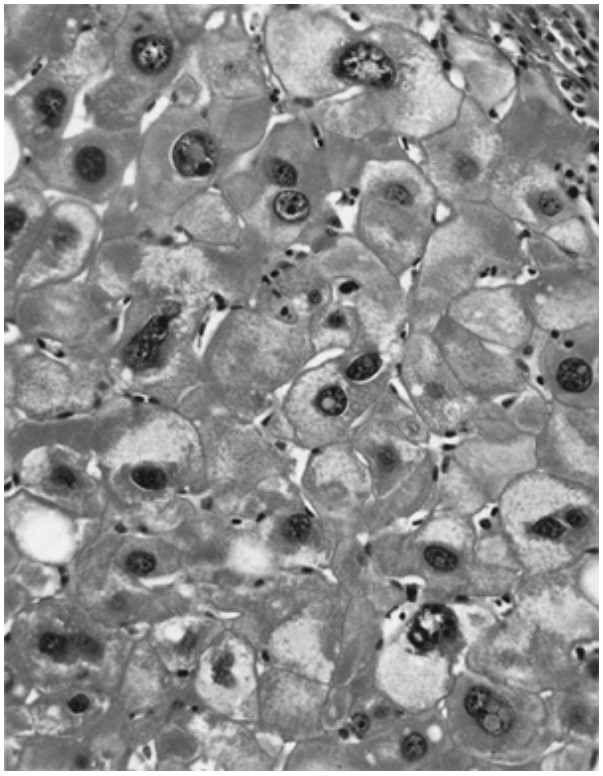
alteration of the spatial relationship between the portal vessels and central veins is typical. Micronodular cirrhosis is less difficult to establish by needle biopsy than is macronodular cirrhosis because the diameter of the biopsy needle usually exceeds that of the small cirrhotic nodules. The capsule of the liver in many noncirrhotic patients is thickened by an increase in fibrous tissue, vessels, and ductules. A small biopsy specimen from such an area (particularly a superficial wedge biopsy) should be interpreted with caution and not diagnosed as cirrhosis.

The morphologic approach in cirrhosis should include an assessment of whether the cirrhosis is fully developed or incomplete, the basic morphologic type (i.e., micronodular, macronodular, or mixed), the degree of activity, and the presumptive cause, if possible. Biopsy specimens showing occasional nodules or extensive fibrosis may be judged to represent early or incomplete cirrhosis, but the designation cirrhosis should be reserved for those with complete loss of acinar architecture. An assessment of the activity should take into account the degree of hepatocellular degeneration and necrosis and the amount of inflammation in the parenchyma of the nodules.

Every effort to establish the underlying cause should be made, although this is not always possible. An etiologic diagnosis can sometimes be established by changes observed in hematoxylin and eosin–stained sections alone (e.g., absence of bile ducts and chronic cholestasis indicating biliary cirrhosis, including cirrhosis secondary to PBC or primary sclerosing cholangitis). Special stains, however, are an important auxiliary technique. Particularly useful are copper stains for Wilson disease and PBC, the PAS stain and immunostains for α_1-antitrypsin deficiency, immunostains of the antigens of hepatitis B, and an iron stain for hemochromatosis.

Putative preneoplastic lesions may be found in cirrhotic livers. Large cell change (liver cell dysplasia) is characterized by nuclear and cytoplasmic enlargement, nuclear hyperchromasia, prominent nucleoli, and occasionally, multinucleation (Fig. 9.105) (144). *Dysplastic nodule* is the term used for grossly or radiologically distinctive nodules that are usually larger than the surrounding cirrhotic nodules and that may differ in color or texture (145). These are classified microscopically as low-grade dysplastic nodules when there are minimal atypical histologic features. They are classified as high-grade dysplastic nodules when there are atypical features in the hepatocytes of the nodule, such as cytoplasmic basophilia, high nuclear/cytoplasmic ratios, nuclear irregularity, and hyperchromasia. High-grade dysplastic nodules often have ill-defined nodules within the large nodule ("nodule-in-nodule" formation), best recognized by compression of surrounding reticulin fibers and different orientation of the liver plates. These are often composed of smaller than normal hepatocytes with high nuclear–cytoplasmic ratios, a feature termed *small cell change* (or *small cell*

dysplasia) (Fig. 9.106). Evidence suggests that this lesion, rather than large cell change, is more likely the precursor of hepatocellular carcinoma in the cirrhotic liver.



▪ **Figure 9.105** Large cell change (liver cell dysplasia) in cirrhosis. Dysplastic cells are large and contain large dark-stained and sometimes irregular nuclei.

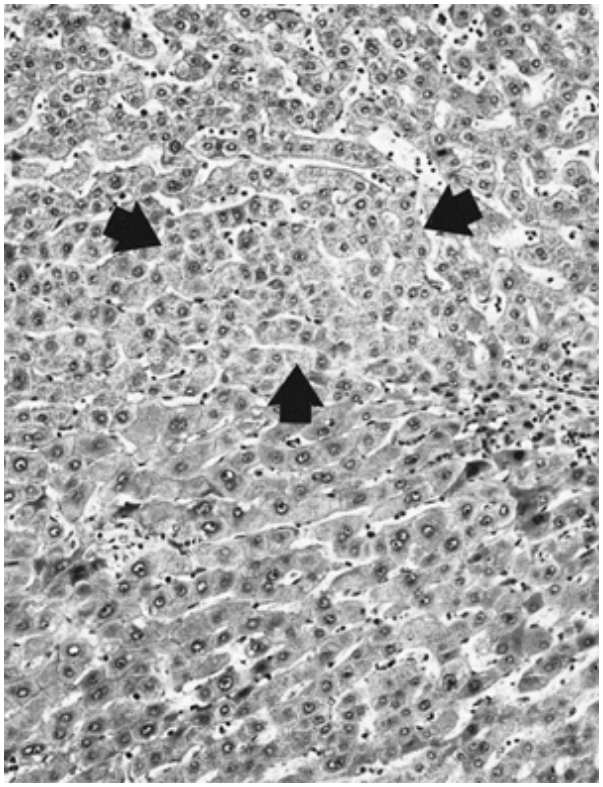
Fibropolycystic Diseases

Lesions and types of cysts

Von Meyenburg complexes or biliary microhamartomas are a common developmental anomaly in the liver (146,147). They are typically found adjacent to normal portal areas and consist of a fibrous stroma that contains several irregular duct-like structures lined by biliary epithelium (Fig. 9.107). These are often somewhat dilated and some may be large enough to be considered cysts. They often contain eosinophilic or bile-stained secretions.

Bile duct cysts are usually solitary and lined by a cuboidal biliary-type epithelium. These are considered to be of developmental origin, and at least some arise from cystic dilatation of a von Meyenburg complex, although in most cases, there are no clues to the

exact pathogenesis of the lesion. The cysts typically contain clear fluid, but become infected and contain pus, or when large, there may be hemorrhage and inflammation secondary to minor trauma.



▪ **Figure 9.106** Small cell dysplasia in a high grade dysplastic nodule. There is a nodular growth of small liver cells with high nuclear/cytoplasmic ratios (*arrows*) within a large cirrhotic nodule.

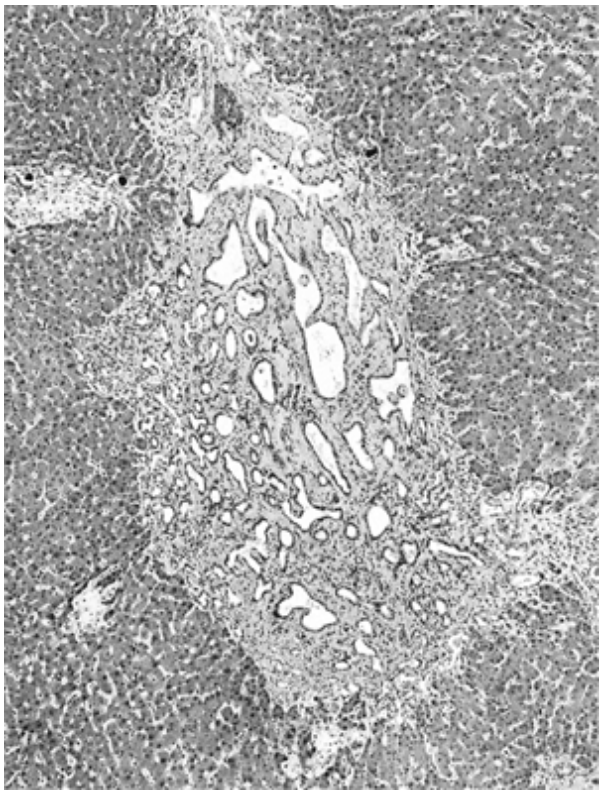
Ciliated foregut cyst is an unusual type of developmental cyst that is lined by ciliated columnar epithelium (148). These cysts are analogous to bronchogenic cysts of the mediastinum. They are extremely rare in the liver.

Ductal plate malformation refers to the persistence of the embryonic ductal plate in the postnatal liver (147). In embryonic life, before the appearance of the acinar bile ducts, the portal tracts are surrounded by a layer of biliary-type cells, termed the *ductal plate*. This structure normally disappears as the true bile duct develops in the center of the portal area. Persistence of parts of the ductal plate may give rise to von Meyenburg complexes, duct-like structures seen in congenital hepatic fibrosis, and cysts of infantile polycystic disease (Fig. 9.108).

Infantile polycystic disease

Infantile polycystic disease, which is an autosomal recessive disease, is

part of a spectrum of lesions that includes infantile polycystic kidney disease, congenital hepatic fibrosis, and Caroli's disease (149). Expression varies from individual to individual. The liver in childhood polycystic disease is enlarged and firm, but cysts are not usually visible grossly. Microscopic sections show numerous irregular duct-like structures in the portal areas. These have apparent branching and irregular angulated extensions into the acini (Fig. 9.108), and there is often a circular arrangement of the ducts, complete or interrupted, characteristic of the ductal plate malformation. In contrast to congenital hepatic fibrosis, relatively little fibrous tissue is present. The ducts are slightly dilated, but true cysts are rare. They are lined by a simple, low columnar to cuboidal epithelium.



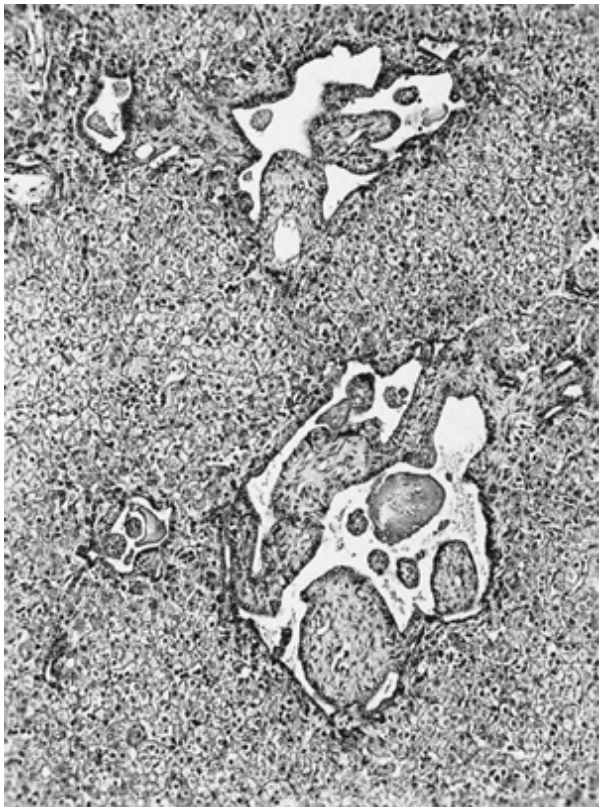
▪ **Figure 9.107** A von Meyenburg complex, which has a dense stroma containing irregular small duct-like structures, a few of which are slightly dilated.

Congenital hepatic fibrosis

In congenital hepatic fibrosis, thick collagenous bands form an extensive network, usually continuous, that links adjacent portal tracts (Fig. 9.109). Numerous bile duct–like structures, some slightly irregular and dilated, are situated in the fibrous septa and sometimes contain mucin or bile. Although they resemble bile ducts, they actually

represent ductal plate remnants. Portal vein branches often appear reduced in size and number, and the sparsity of venous channels might account in part for the portal hypertension. The irregular intervening parenchyma frequently has a "jigsaw"

pattern reminiscent of that seen in biliary cirrhosis. However, unlike cirrhosis, congenital hepatic fibrosis does not show evidence of parenchymal destruction or regeneration. The hepatocytic plates are regular and one cell in thickness, and there is an abrupt transition between the normal-appearing hepatocytes and the collagenous septa.

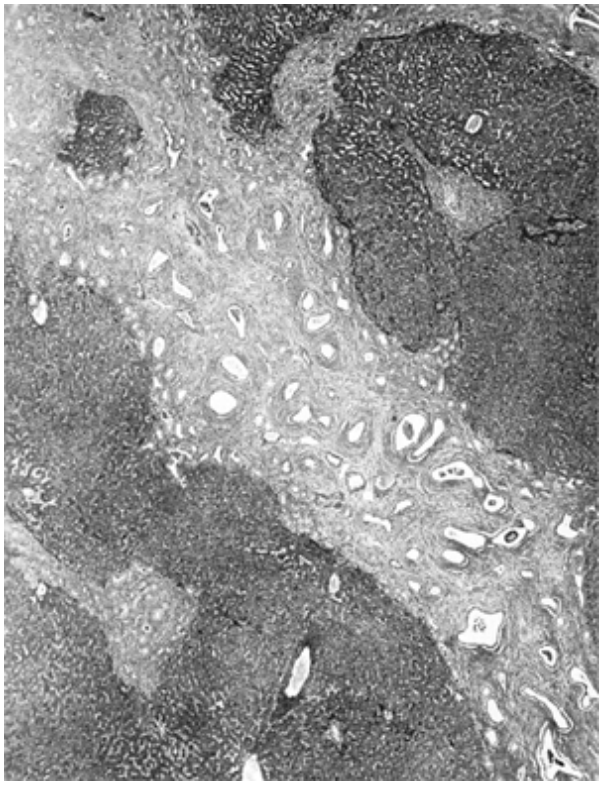


▪ **Figure 9.108** Infantile polycystic disease. The ducts are lined by cuboidal epithelium, show polypoid intraluminal projections, and are for the most part empty. They arise from remnants of the embryonic ductal plate.

Adult polycystic disease

Adult polycystic disease is frequently associated with adult polycystic kidney disease. Numerous cysts, varying from less than 1 mm to more than 12 cm in diameter, are present, containing clear, colorless or straw-colored fluid, unless infected. Microscopically, the cysts appear to originate in the portal areas. They are lined by low columnar to cuboidal

epithelium (Fig. 9.110) and have a collagenous supporting stroma that can be infiltrated by a few inflammatory cells. Von Meyenburg complexes (biliary microhamartomas) are frequently present, and components of the complexes sometimes lie adjacent to the cysts, suggesting that the cysts evolve from progressive dilatation of the biliary channels in the complexes. Occasional cases show many von Meyenburg complexes and no cysts.



▪ **Figure 9.109** Congenital hepatic fibrosis. This Masson stain shows islands of parenchyma separated by irregular bands of fibrous tissue that contain numerous small ductal plate remnants.

Tumors

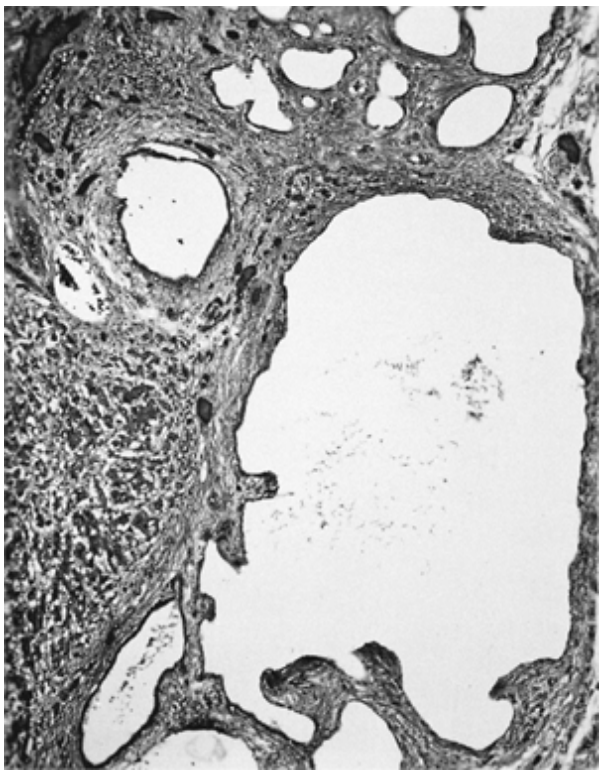
Hepatocellular tumors

Hepatocellular adenoma. This is composed of benign hepatocytes arranged in sheets and cords without an acinar architecture (Fig. 9.111) (150). It is easily mistaken for normal liver if the absence of portal areas is not noticed. The tumor cells are the same size or slightly larger than non-neoplastic hepatocytes and often have a pale cytoplasm because of an increased glycogen and/or fat content. The nuclei are uniform and regular, and the nuclear–cytoplasmic ratio is normal; mitoses are almost never seen. Thin-walled vascular channels are

scattered throughout the tumors, but large arteries are only seen around the periphery. The sinusoids are usually compressed with flattened lining cells, contributing to the sheet-like appearance. Sometimes the sinusoids are dilated, a finding that has mistakenly been called peliosis. Kupffer cells are present but usually inconspicuous, and hematopoietic cells may also be found in sinusoids.

Focal nodular hyperplasia. This is usually a solitary nodule with a typical gross appearance that is extremely useful in making the diagnosis (150). The lesions are well circumscribed but unencapsulated. When on the surface of the liver, they may appear umbilicated. They

are usually of a lighter color than the surrounding liver, ranging from yellow to tan or light brown. The cut surface typically contains a central "stellate" scar with radiating fibrous septa dividing the lesion into nodules.

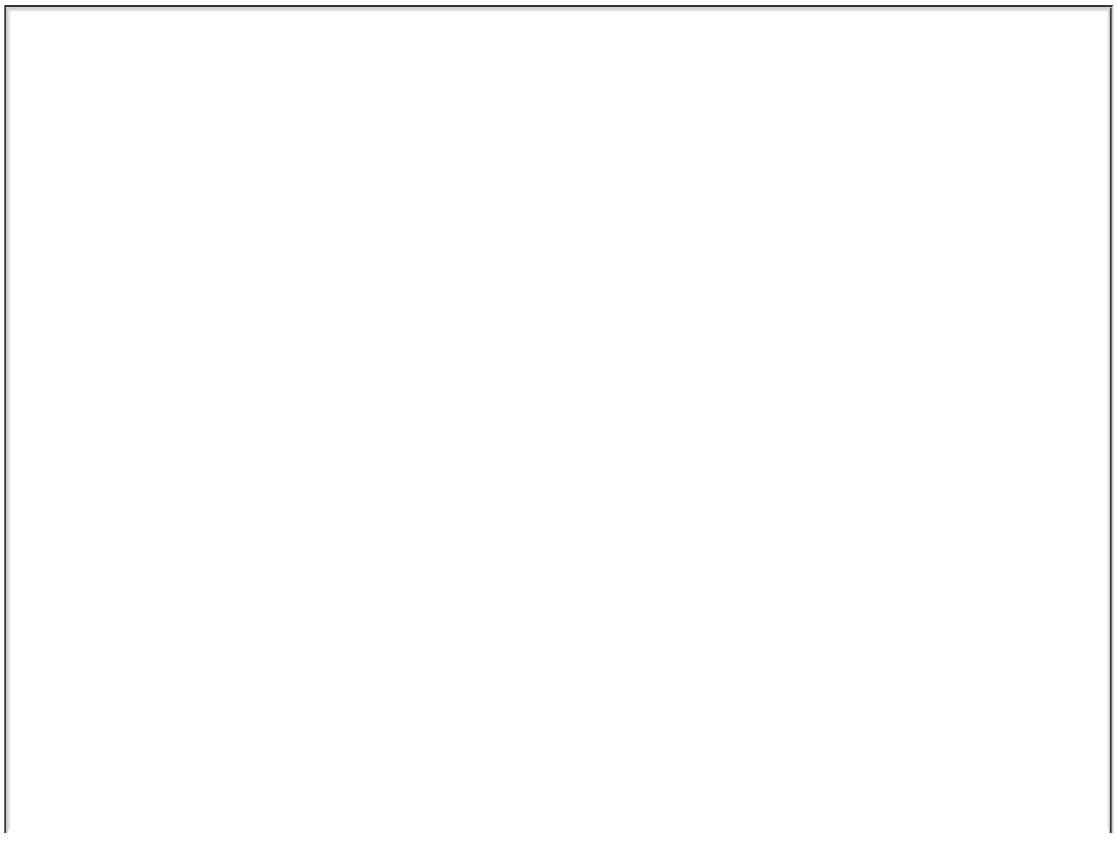


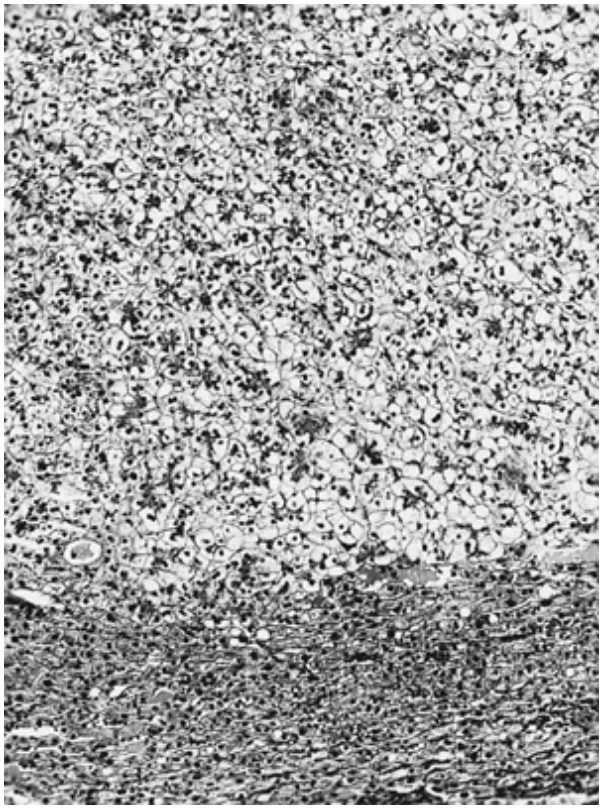
▪ **Figure 9.110** Adult polycystic disease. Multiple cysts of varying size are lined by cuboidal to flattened epithelium.

The microscopic features correspond to the gross pathology. A section through the center of the lesion nearly always shows the central "stellate" scar that usually contains one or more large arteries, often with abnormal intimal or medial fibromuscular proliferation (Fig. 9.112). Proliferating ductules are usually present but true bile ducts are

lacking. Fibrous septa of variable size radiate from the central scar. Between the septa are hyperplastic nodules of normal hepatocytes with cholestatic features, such as cholate stasis, and copper storage, and even bile pigments are usually present to some degree and are occasionally prominent, making the lesion resemble a focal area of biliary cirrhosis. A needle biopsy is easily misinterpreted as cirrhosis unless one is aware that it is from a solitary lesion, but the large artery in an area of scarring provides a valuable clue to the diagnosis.

Hepatocellular carcinoma. The cells of hepatocellular carcinoma resemble normal liver cells to a variable extent (151). In some tumors, the cells are so well differentiated that they are difficult to distinguish from normal hepatocytes or from the cells of hepatocellular adenoma. At the other extreme are tumors with cells that are anaplastic and poorly differentiated, showing only minimal evidence of liver cell origin. Most tumors, however, show definite evidence of hepatocellular differentiation. The tumor cells have distinct cell membranes and an eosinophilic, finely granular cytoplasm. Bile canaliculi are usually present between cells (Fig. 9.113) and, although sometimes hard to find, can usually be seen by light microscopy. Immunostains for CEA are useful for demonstrating canaliculi because of a CEA cross-reacting substance called *biliary glycoprotein I (BGP-1)* located in the canalicular membrane. Bile pigment may be present in tumor cells or in dilated canaliculi and is the most helpful microscopic feature in establishing the diagnosis. The tumor cell nuclei are usually large, producing a high nuclear–cytoplasmic ratio. They show variable degrees of anaplasia and usually have prominent nucleoli.

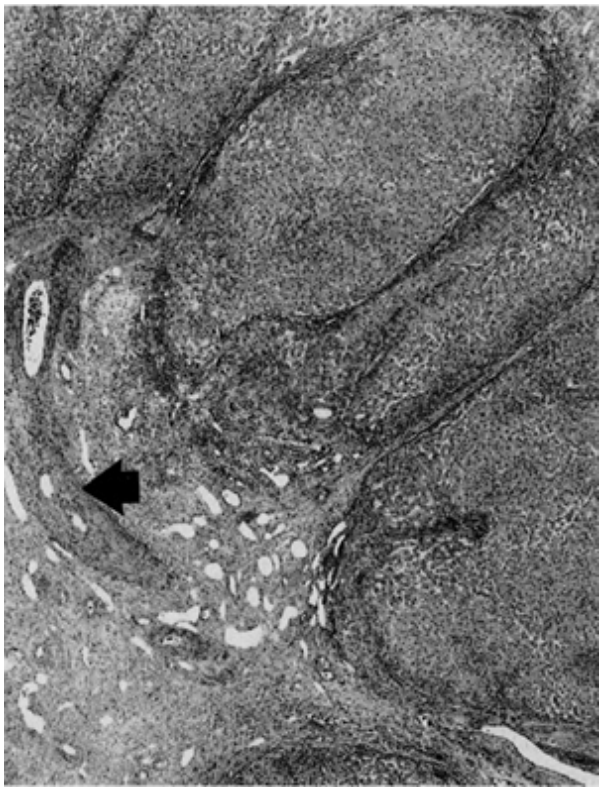




▪ **Figure 9.111** Hepatocellular adenoma, composed of a sheet-like growth of large, pale hepatocytes without acinar architecture. Compressed normal liver tissue is present in the lower part of the picture.

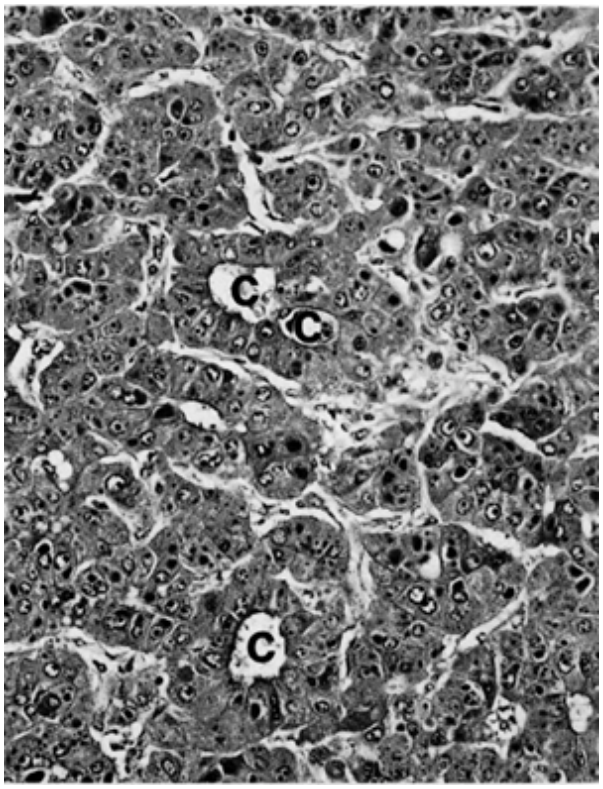
Several histologic growth patterns may be found in hepatocellular carcinoma, and because the cytologic features can be so variable, recognition of one of these patterns can be helpful in arriving at a diagnosis. Most frequent is the trabecular pattern (Fig. 9.114) in which the tumor cells grow in thick cords that attempt to recapitulate the cell plate pattern

of the normal liver. The trabeculae are separated by vascular spaces (sinusoid-like) with very little or no supporting connective tissue. Sometimes the centers of the trabeculae contain dilated canaliculi, producing a pseudoglandular pattern, while solid patterns are produced when the trabeculae grow together, forming sheets of tumor cells.



▪ **Figure 9.112** Focal nodular hyperplasia. A section through the central scar shows an abnormally thickened large artery (*arrow*). Cirrhosis-like nodules surround the central scar.

Fibrolamellar hepatocellular carcinoma. This is usually considered to be a histologic variant of hepatocellular carcinoma, but there is considerable evidence that it is actually a completely different biologic entity, occurring in a different population with a better prognosis than the other types of hepatocellular carcinoma. The pathologic features of fibrolamellar carcinoma are quite distinctive (152). Microscopically, these tumors appear to be well-differentiated hepatocellular carcinomas but instead of trabeculae separated by sinusoids, they are composed of sheets of large polygonal tumor cells separated by abundant collagen bundles arranged in parallel lamellae (Fig. 9.115), hence the name *fibrolamellar*. The tumor cells have a characteristic cytologic appearance with cytoplasm that is deeply eosinophilic and granular due to the presence of numerous mitochondria. Approximately 50% of these tumors have cytoplasmic "pale bodies" that represent intracellular fibrinogen storage.



▪ **Figure 9.113** Moderately differentiated hepatocellular carcinoma with several dilated canaliculi (*C*) easily visible between tumor cells.

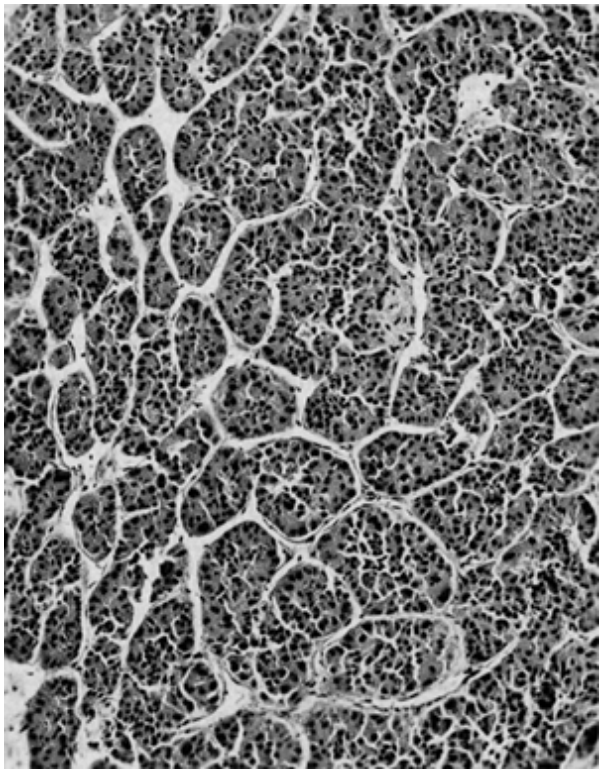
Hepatoblastoma. Epithelial hepatoblastoma is composed of fetal- or embryonal-type liver cells, or both (153). Tumors with predominantly fetal cells mimic fetal liver with a distinctive light-and-dark cell pattern and foci of hematopoiesis (Fig. 9.116). Embryonal cells are smaller and more basophilic with a high nuclear–cytoplasmic ratio. They tend to form acini, tubules, or papillary structures. Mixed epithelial–mesenchymal hepatoblastoma has an epithelial component of either fetal or embryonal cells and also a mesenchymal-like element that may consist of primitive mesenchyme, osteoid (with or without calcification), and rarely cartilage or rhabdomyoblasts. An anaplastic type (small cell undifferentiated) and a macrotrabecular type (hepatocellular carcinoma-like) of hepatoblastoma have also been recognized.

Biliary tumors

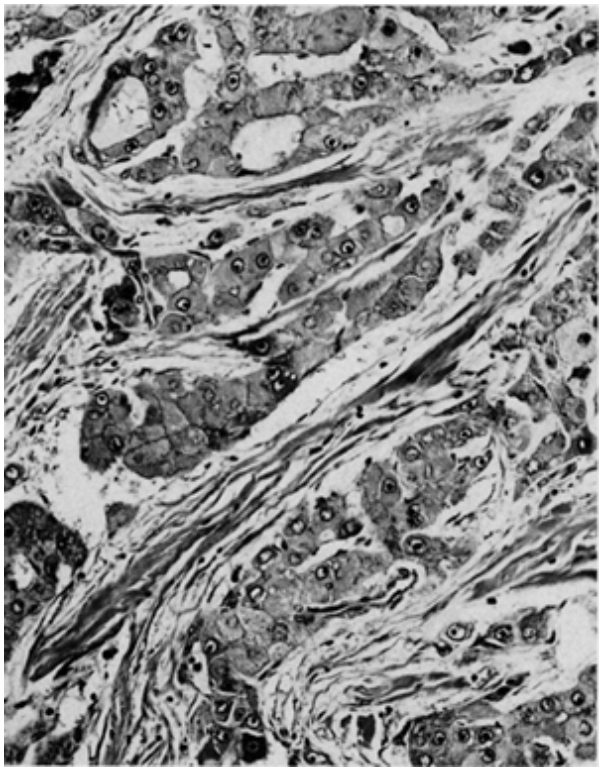
Bile duct adenoma (peribiliary gland hamartoma) is usually a solitary subcapsular nodule, although occasional livers have more than one nodule. The lesions may be up to 4 cm in diameter, but 90% are 1 cm or less. They are composed of a proliferation of small, round, normal-appearing ducts with cuboidal, slightly basophilic cells that have very

regular nuclei and lack any evidence of dysplasia or mitotic figures (Fig. 9.117). There is

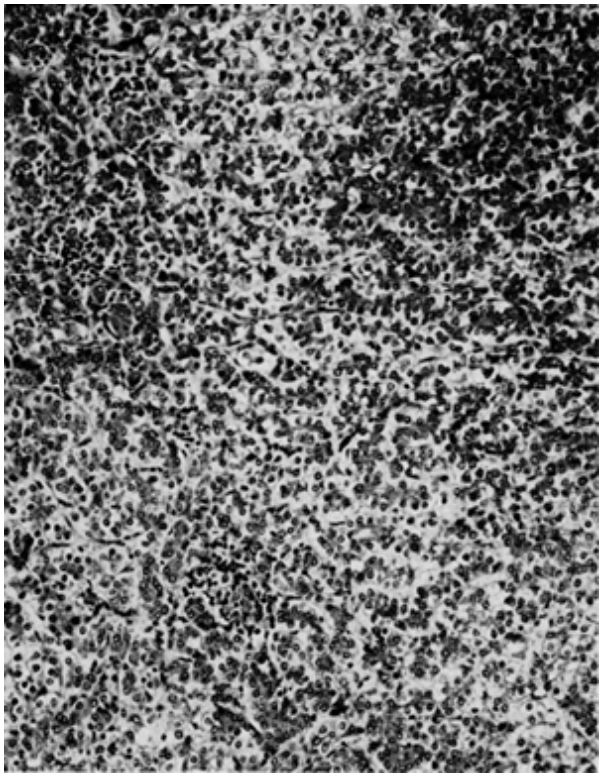
always a fibrous supporting stroma that may be dense and hyalinized. Preexisting normal or inflamed portal areas may be present within the tumor. Although these lesions have traditionally been regarded as benign tumor of bile ducts, recent studies have shown the tumor cells to have the immunophenotype of normal peribiliary glands, indicating that they are actually peribiliary gland hamartomas (154).



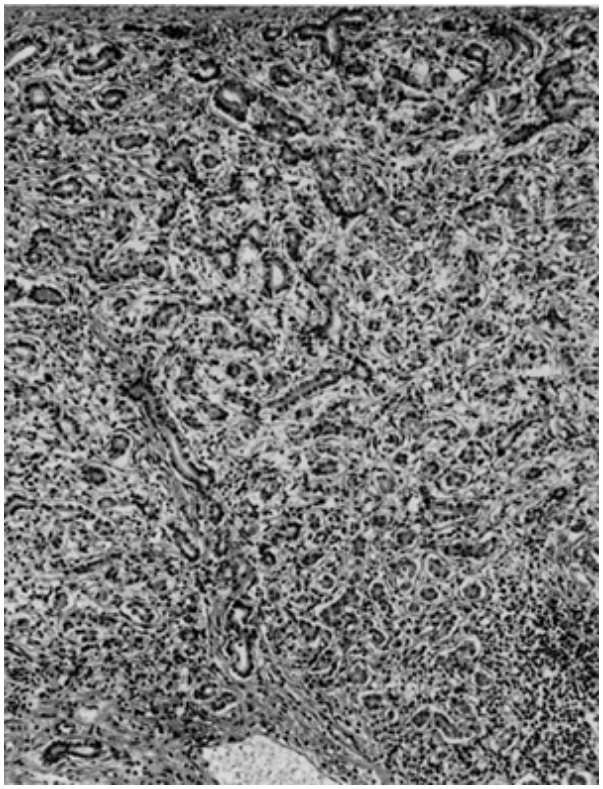
▪ **Figure 9.114** Trabecular growth pattern of hepatocellular carcinoma. The tumor cells form thick cords (resembling islands of cells in cross section), separated by vascular spaces mimicking hepatic sinusoids.



▪ **Figure 9.115** Fibrolamellar hepatocellular carcinoma. The tumor cells are large and polygonal and (in contrast to the trabecular hepatocellular carcinoma) are embedded in a fibrous stroma arranged in parallel lamellae.

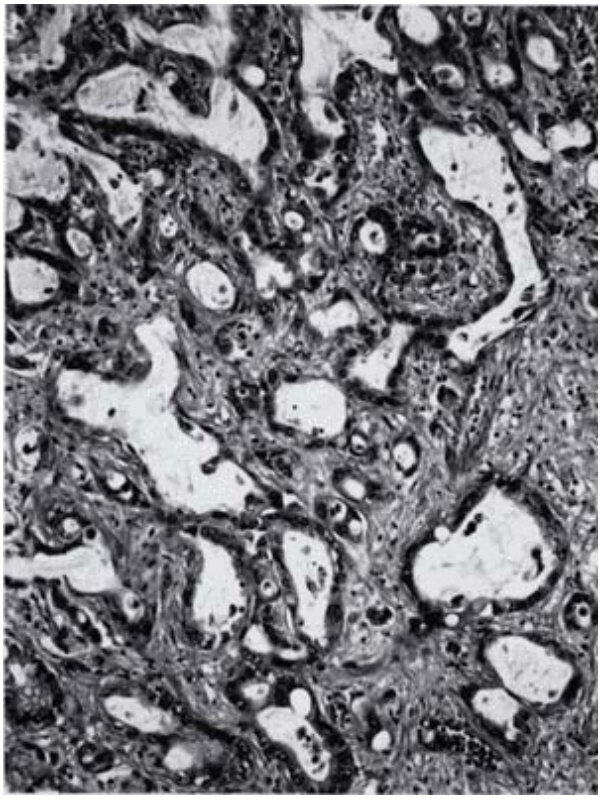


▪ **Figure 9.116** Fetal hepatoblastoma mimics fetal liver with a distinctive "light-and-dark" cell pattern and small clusters of hematopoietic cells.



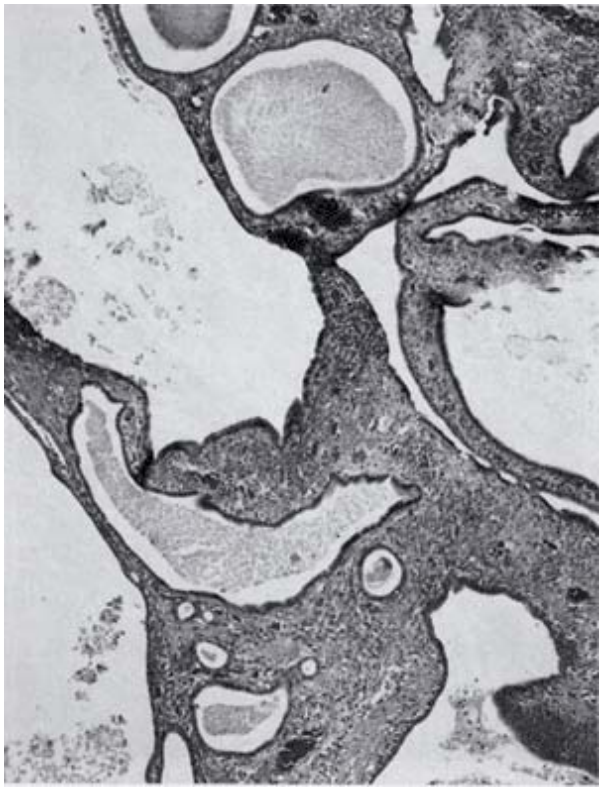
▪ **Figure 9.117** Bile duct adenoma (peribiliary gland hamartoma). The tumor is a localized proliferation of small benign glands in a fibrous stroma.

Cholangiocarcinoma. Microscopically, these resemble adenocarcinomas arising in other parts of the body (155). They are glandular carcinomas (Fig. 9.118) composed of cells resembling biliary epithelium. The cells are low columnar or cuboidal with slightly basophilic cytoplasm and nuclei that are smaller than those of hepatocellular carcinoma; nucleoli are inconspicuous. Mucin can often be demonstrated by special stains but is seldom abundant. There is typically a dense fibrous stroma, which can be helpful in their distinction from hepatocellular carcinoma but not from metastases. Calcifications can sometimes be seen in the fibrous tissue. The tumors are usually well-differentiated gland-forming neoplasms, but they may be poorly differentiated, papillary, or solid, displaying the full range of appearances that can be seen in adenocarcinomas. Occasional tumors show focal squamous differentiation (adenosquamous or mucoepidermoid carcinoma). There are no reliable histologic features distinguishing intrahepatic cholangiocarcinoma from metastatic adenocarcinoma. The diagnosis depends on the reasonable exclusion of an extrahepatic primary.



▪ **Figure 9.118** Cholangiocarcinoma can resemble an adenocarcinoma arising anywhere in the body. There is typically a dense fibrous stroma infiltrated by irregular glands composed of pleomorphic tumor cells.

Biliary cystadenoma and cystadenocarcinoma. These are multilocular cystic neoplasms that arise from intra- and extrahepatic bile ducts (155). Developmental cysts are never truly multilocular (although secondary changes occasionally make them seem so). Cystadenomas and cystadenocarcinomas may occur anywhere in the intra- or extrahepatic bile ducts, but nearly all are partly or totally within the liver. Most are over 10 cm in diameter. Microscopically, biliary cystadenoma has a mucin-secreting columnar epithelium lining the cysts (Fig. 9.119). The lining cells have a pale eosinophilic cytoplasm and basally oriented nuclei, typical of biliary-type epithelium. The epithelium is supported by what has been called a *mesenchymal stroma*. This is compact and cellular, and resembles the stroma of the ovary. Biliary cystadenoma is regarded as a premalignant tumor, and when malignancy develops, it is called *cystadenocarcinoma*. There may only be in situ carcinoma with papillary growth into the cysts, or there may be frank invasive adenocarcinoma.



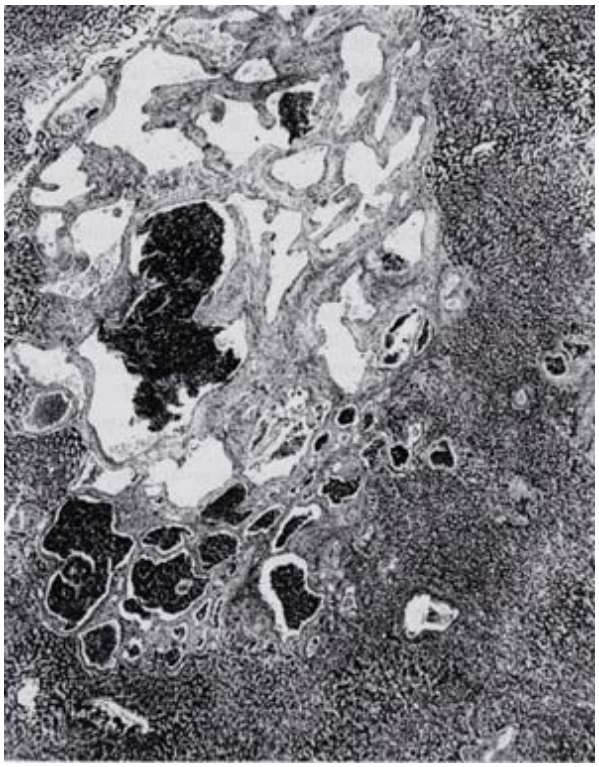
▪ **Figure 9.119** Cystadenoma is a multilocular cystic neoplasm with mucinous epithelium overlying a compact mesenchymal stroma.

Biliary papillomatosis is an extremely rare disease in which multiple benign papillary adenomas, similar to adenomas of the intestinal tract, arise in the bile ducts (154). As with intestinal adenomas, invasive carcinoma may develop.

Hemangiomas and Other Vascular Tumors

Hemangiomas are the only common vascular tumors of the liver. Most are less than 4 cm in diameter, but occasional tumors may be as large as 30 cm. Microscopically these are cavernous hemangiomas with varying-sized vascular channels (Fig. 9.120) lined by flattened endothelial cells (156). They are usually discrete and well demarcated from the surrounding liver, although an occasional hemangioma may contain trapped bile ducts or foci of parenchyma. Variable amounts of fibrous tissue separate the vascular channels. Many consist of thin, delicate strands, while others have large areas of scarring. Fresh and organizing thrombi may be found in the vascular channels. The dynamics of these thrombi are not known, but they are commonly observed in surgically resected hemangiomas. Because of the sluggish blood flow through these tumors, small thrombi are probably constantly

forming and lysing, contributing to the typically heterogeneous appearance by MRI. Fibroblasts can be found growing into a few thrombi and are probably the source of the scarring that results in the "sclerosing hemangioma." In end-stage sclerosed and/or calcified hemangiomas, an underlying vascular pattern can usually still be discerned, providing the clue to the diagnosis.

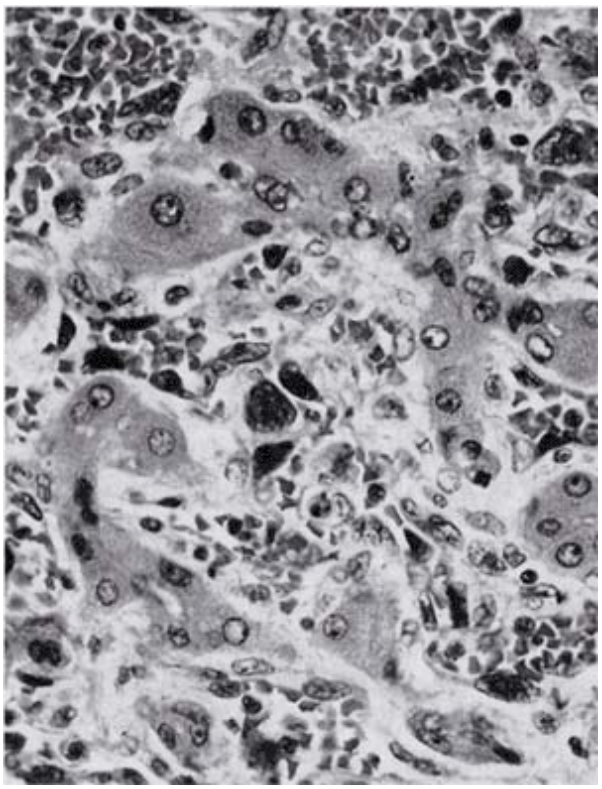


▪ **Figure 9.120** Hemangioma consists of a fibrous stroma containing "cavernous" blood-filled spaces lined by flattened endothelial cells.

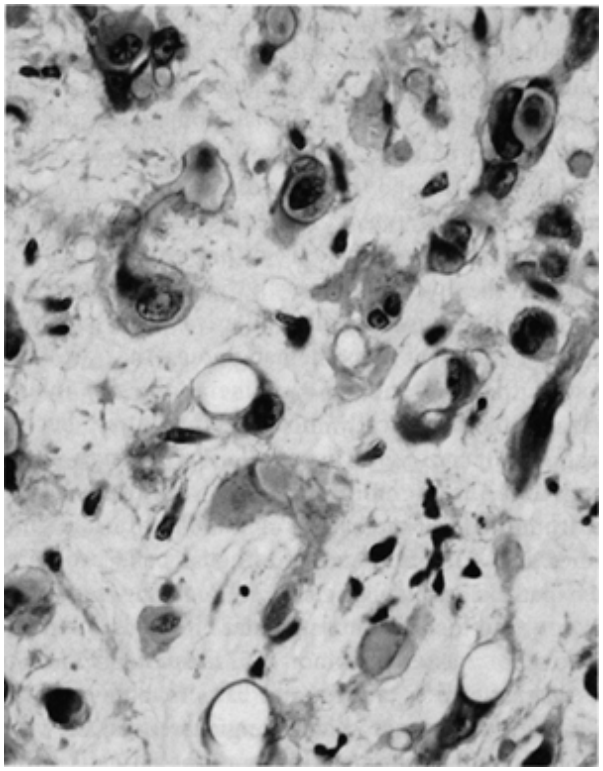
Other vascular tumors are exceedingly rare. *Infantile hemangioendothelioma* is a rare tumor that can occur in the liver in infants (156). They consist of small proliferating capillary-like vascular channels, similar to the capillary hemangiomas that are common in the skin and mucous membranes of infants. Although histologically benign, they may become large enough to cause hepatic failure or high-output congestive heart failure due to shunting through the tumor. *Angiosarcoma* is a rare highly malignant tumor in which atypical endothelial cells proliferate in hepatic sinusoids (Fig. 9.121), causing hepatocyte atrophy and formation of vascular channels and sometimes solid masses of tumor (157). *Epithelioid hemangioendothelioma* is an equally rare malignant tumor (although not so aggressive as angiosarcoma), in which plump, epithelioid-appearing endothelial cells

proliferate in the hepatic vasculature, producing a dense fibrous stroma (Fig. 9.122), similar to that seen in cholangiocarcinoma and metastatic adenocarcinoma (157).

Many of these are misdiagnosed as adenocarcinoma or, if the tumor cells are few, as a benign lesion.



▪ **Figure 9.121** Angiosarcoma is a proliferation of malignant endothelial cells that fills the hepatic sinusoids, causing the hepatocytes to atrophy.



▪ **Figure 9.122** Epithelioid hemangioendothelioma is a malignant tumor that forms intracellular capillary lumina and typically produces a dense fibrous stroma as it fills the vascular spaces.

Metastases

Metastases far outnumber primary liver tumors, so that any liver mass is more likely to be metastatic than primary. Only if a tumor is benign or shows clear evidence of hepatocellular differentiation, indicating hepatocellular carcinoma, can it be confirmed that it is primary in the liver. Any other malignancy, particularly an adenocarcinoma, should be presumed to be metastatic. Microscopically, metastases usually resemble the primary tumor. If the primary lesion is known and has been biopsied or excised, comparison of the microscopic appearance of the tumor in the liver with that of the primary will confirm whether the hepatic tumor is a metastasis.

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Chapter 10
Mechanisms of Liver Injury

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Gregory J. Gores

Key Concepts

- Apoptosis is a prominent feature of acute and chronic liver disease.
- Various cell types in the liver can be affected by injurious stimuli.
- Death receptor-mediated, mitochondria-dependent cell death is common in liver injury.
- Apoptosis leads to inflammation and injury in acute liver diseases, and in chronic liver diseases sustained apoptosis begets fibrosis.
- Manipulation of apoptotic pathways has wide therapeutic potential.

The liver is in constant cellular flux with careful removal of senescent or damaged cells and controlled repopulation through a progenitor cell compartment. The unique juxtaposition to the intestine, large resting blood flow, and unfiltered contact with portal blood also provides the liver with a unique sensitivity to various gut-derived, diet-derived, or blood-borne insults. Under basal conditions, removal of hepatocytes occurs mainly through apoptosis without reactive inflammation. Liver injury, on the other hand, is associated with cell death, inflammation, and regeneration.

Cell death in the liver can be apoptotic or necrotic or a combination of the two. Historically, apoptosis, or programmed cell death, has been viewed as a carefully choreographed cascade resulting in protease and endonuclease activation. It has been defined morphologically on the basis of characteristic nuclear appearance, the generation of membrane-bound apoptotic bodies (Councilman bodies), and the absence of an inflammatory reaction. Necrotic cell death has been considered the antithesis of apoptotic cell death. It has been defined on the basis of cellular energy depletion, characteristic morphology of cytoplasmic vacuolation, cell swelling, and the presence of an inflammatory reaction. Cell swelling, a cardinal feature of necrosis, results from an inability to maintain ion gradients, such that the process is in fact called *oncotic necrosis*. Over the last few years, with better understanding of both pathways of cell death, these arbitrary morphologic definitions have become less important. It is recognized that both apoptosis and necrosis can be triggered by the same stimulus and occur in the same disease process. Apoptosis has been shown to be a prominent feature of several liver diseases (Fig. 10.1). The dogma has shifted from apoptosis being a physiologic, bland, noninflammatory process to one that is pathologic, occurs in disease processes, and causes liver inflammation, injury, and fibrosis (Fig. 10.2). This

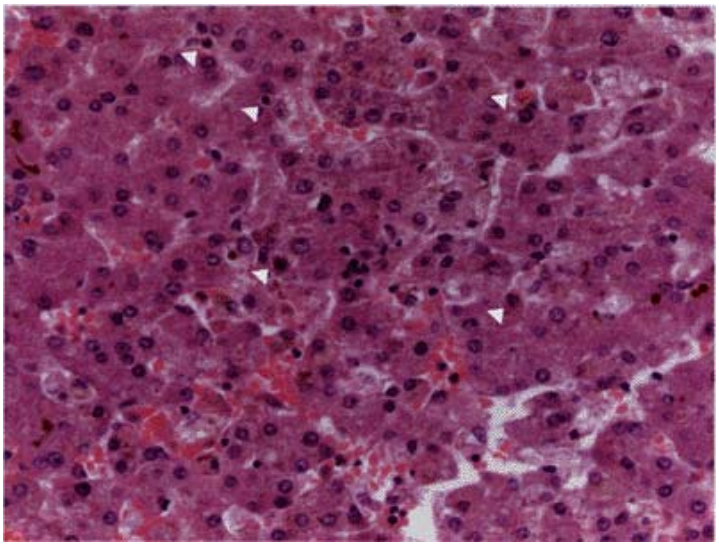
chapter discusses mechanisms of liver injury. It is divided into two sections. The first section provides a general overview of mechanisms of death in the liver. Apoptosis and necrosis are both discussed with emphasis on death receptors (DRs) and the role of mitochondria in cell fate. The second section focuses on liver injury in some common disorders.

Basic Mechanisms

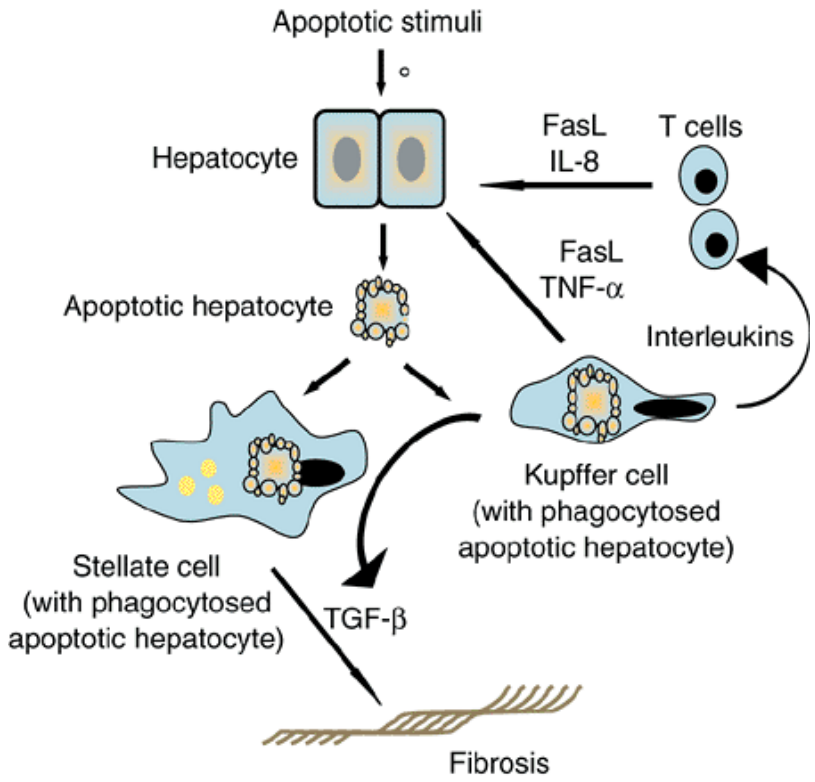
Apoptosis

Liver apoptosis is a prominent feature of most liver disorders, including drug-induced liver disease, viral liver disease, alcoholic liver disease, nonalcoholic liver disease, cholestatic liver disease, and vascular liver disease. Apoptosis of hepatocytes, Kupffer cells, sinusoidal endothelial cells (SECs), hepatic stellate cells (HSCs), and cholangiocytes has been observed in different liver diseases (1,2,3,4,5,6). The initiation of apoptosis occurs by

two fundamental pathways: (i) The DR or extrinsic pathway and (ii) the mitochondrial or intrinsic pathway (7). The extrinsic pathway is triggered by ligation or oligomerization of DRs. The mitochondrial pathway is activated by several intracellular perturbations, including deoxyribonucleic acid (DNA) damage, lysosomal permeabilization, endoplasmic reticulum (ER) stress, chemotherapeutic agents, oxidative stress, toxins, and sustained increases in Ca^{2+} (8). Cells that die through the extrinsic pathway are classified as type I or type II cells. In type I cells, amplification and conduction of death signals occurs exclusive of mitochondrial involvement. In type II cells, DR activation is not sufficient to propagate a lethal signal without mitochondrial involvement. Hepatocytes are type II cells and have an obligate need for mitochondria in cell death (9).



▪ **Figure 10.1** Hepatocyte apoptosis in human liver. Photomicrograph of a hematoxylin–eosin–stained section from the liver of a patient with primary sclerosing cholangitis shows several apoptotic hepatocytes (*white arrow heads*). Condensed nuclei (pyknosis) surrounded by an eosinophilic cytoplasm are seen.



▪ **Figure 10.2** Hepatocyte apoptosis promotes fibrosis. The link between hepatocyte apoptosis and fibrosis is illustrated here. Apoptosis is the proximal event in hepatic fibrosis. Apoptotic hepatocytes are engulfed by stellate cells, leading to their activation, and by Kupffer cells, leading to the secretion of several proinflammatory and proapoptotic cytokines. Kupffer cell–derived cytokines promote stellate cell activation and further promote apoptosis. Activated stellate cells play a key role in fibrosis. IL-8, interleukins-8; TNF-α, tumor necrosis factor α; TGF-β, transforming growth factor β.

In a homeostatic setting, apoptosis is accompanied by activation of phagocytosis, leading to efficient removal of cellular corpses without damage to healthy cells (10). However, apoptosis, if massive or simultaneous, results in liver injury. For example, in the setting of massive apoptosis, such as fulminant hepatic failure (FHF), successful hepatocyte repopulation leads to recovery (11). In such an environment, if hepatocyte repopulation is protracted or delayed, liver injury will result. Apoptosis is harmful not only in a passive sense of inadequate repopulation but also as an active inducer of inflammatory processes. In fact, recent data have established a mechanistic link between liver apoptosis, injury, inflammation, and fibrosis in chronic liver disease (12). Apoptotic body engulfment by phagocytic cells leads to their activation. This results in chemokine secretion, recruitment of leukocytes and inflammatory cells to the liver, and amplification of liver injury. HSCs are activated by phagocytosis of apoptotic

bodies as well, leading to fibrosis (13) (Fig. 10.2).

Death receptors and the liver

DRs, transmembrane proteins that belong to the tumor necrosis factor (TNF)/nerve growth factor superfamily,

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are essential for ligand–mediated cell death. There are several known death receptors: Fas (CD95/Apo-1), tumor necrosis factor receptor 1 (TNFR1), tumor necrosis factor receptor 2 (TNFR2), tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) TRAIL receptor 1 (TRAIL-R1/DR 4), TRAIL receptor 2 (TRAIL-R2/ DR 5/Killer/ TRICK2), DR 3 (DR 3/Apo-3/TRAMP/WSL-1/LARD), and DR 6. Of these receptors, Fas, TNFR1, and TRAIL are thought to be of significance in liver injury. DRs are activated by engagement with their cognate ligands, Fas ligand (FasL), TNF-α, and TRAIL (Figs. 10.3 and 10.4). Interaction

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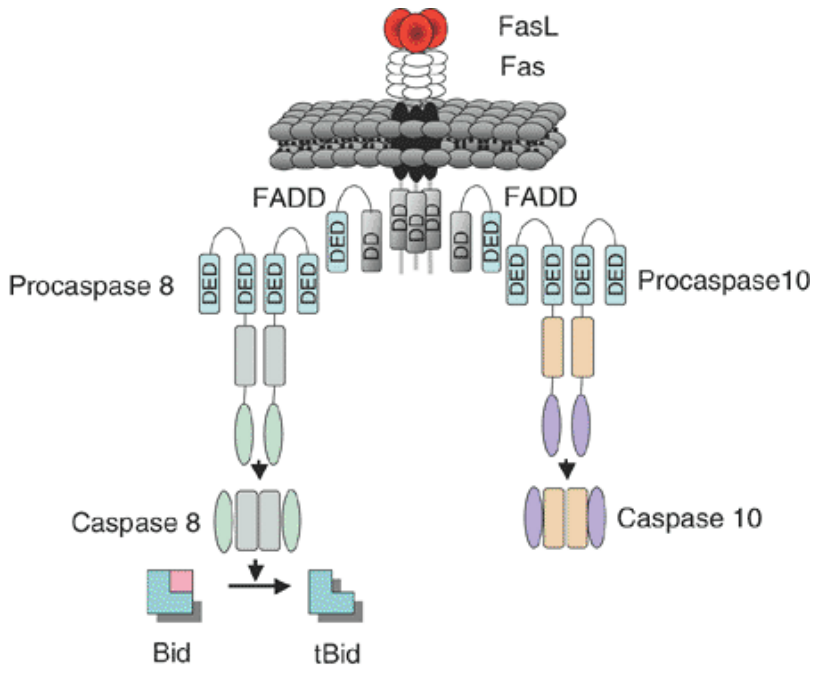
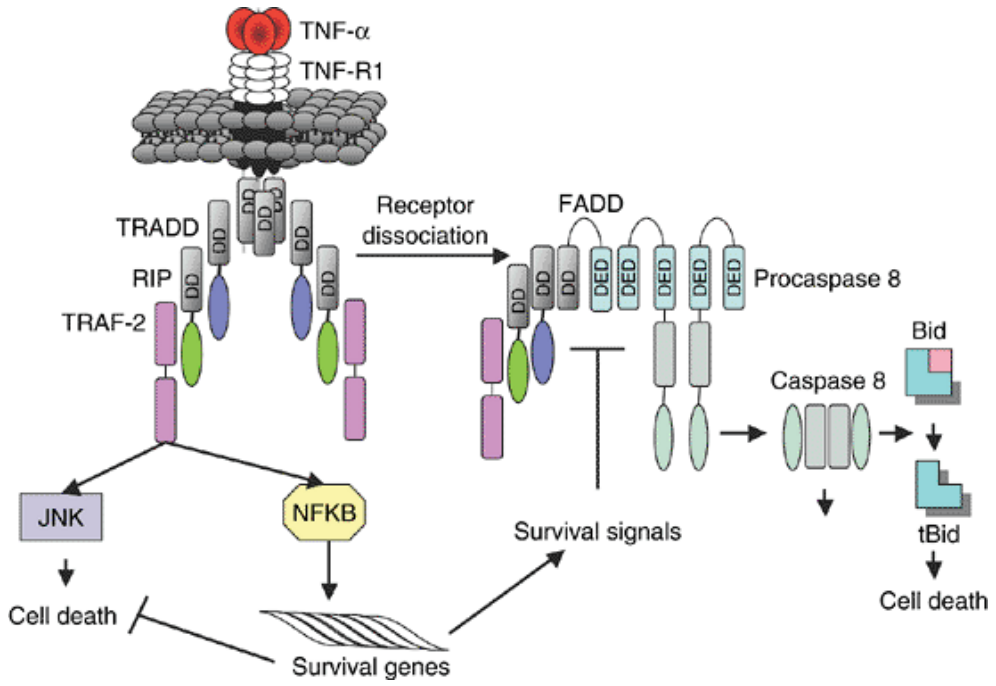
of these ligands with their cognate receptors triggers intracellular signaling pathways. Receptor ligation brings together the intracellular death domains (DDs) that through adaptor proteins (discussed in the following text) lead to the activation of caspase 8 (an initiator caspase). Once activated, caspase 8 cleaves Bid, a cytoplasmic protein. The cleaved protein, tBid, translocates to mitochondria, leading to its release to mitochondrial effectors of apoptosis, ultimately activating caspases 3 and 7 (effector caspases).



▪ **Figure 10.3** Fas receptor and associated signaling. The Fas receptor is activated on ligation with Fas ligand (FasL). The ligated receptor forms homotypic trimers, leading to intracellular signaling initiated by their intracellular death domains (DDs). The DDs of trimerized Fas associate with the protein Fas-associated death domain (FADD) through its DD. FADD in turn possesses death effector domains (DEDs) that bind and activate procaspase 8 by proteolytic cleavage. Caspase 8 leads to the cleavage of Bid to tBid, a form that translocates to the mitochondria and causes

mitochondrial dysfunction. Caspase 10 is also activated by FADD, but its intracellular targets remain undefined. This conglomeration of proteins is known as the *Fas death-inducing signaling complex (DISC)*.



▪ **Figure 10.4** Tumor necrosis factor receptor (TNFR1) and associated signaling. Ligation of tumor necrosis factor α (TNF-α) to its receptor TNFR1 initiates two distinct set of signals. The initial signaling pathway involves the proteins TRADD (TNFR-associated protein with death domain), receptor-interacting protein (RIP), and TRAF-2 (TNF-associated factor 2), leading to the activation of nuclear factor κB (NFκB) and c-jun *N*-terminal kinase (JNK). Following this the receptor undergoes a conformational change, internalization, interaction with FADD, and activation of caspases 8 and 10 with Bid cleavage.

Fas is expressed by every cell type in the liver, including hepatocytes, cholangiocytes, SECs, stellate cells, and Kupffer cells (6,14,15). Activation of Fas, in most instances, requires binding with membrane-bound FasL-expressing cells or soluble FasL. FasL is expressed in cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. This provides an efficient means of removal of unwanted hepatocytes, such as virus-infected hepatocytes and cancer cells, by T lymphocytes (16,17). Mice genetically deficient in Fas exhibit hepatic hyperplasia, proving a role for Fas in hepatic homeostasis in healthy individuals (18). The multifaceted role of Fas in hepatic homeostasis and injury is introduced in this section and discussed in greater detail in the subsequent sections.

Although the significance of Fas in liver disease is undisputed, TNF-α plays a significant, if somewhat complementary, role. There is clear overlap in the

spectrum of liver injury associated with TNF-α and Fas, with some features unique to TNF-α receptor–mediated signaling. TNFR1 and TNFR2 are both expressed on hepatocytes, although only TNFR1 expresses a DD and executes the apoptotic program. TNFR1 activation leads to both survival and death signals. Immediate recruitment of TNF receptor–associated protein 2 (TRAF-2) and receptor-interacting protein (RIP) to ligated TNFR1 leads to the activation of nuclear factor κB (NFκB) (19). This transcription factor activates a variety of survival genes (e.g., *Bcl-XL*, *A1*, *XIAP*, and *cFLIP*). Apoptosis is initiated subsequently through the adaptor protein TRADD-mediated caspase 8–Fas-associated death domain (FADD) activation in a receptor-initiated albeit receptor-independent complex (20,21). Therefore, TNFR1 receptor signaling is complex because it usually leads to survival signals but in pathophysiologic states can induce apoptosis.

TRAIL and its receptors add further complexity to DRs and their role in the liver. TRAIL receptors 1 and 2 induce apoptosis through caspase activation, similar to Fas (9,22), whereas TRAIL receptors 3 and 4 are thought to function as decoy receptors, interfering with TRAIL-induced death signaling (23). Traditionally, TRAIL has been thought of as being harmless to normal hepatocytes but efficiently apoptotic in solid tumors (24,25,26). Recent experiments have demonstrated a role for TRAIL in murine hepatocyte apoptosis in several models in hepatitis (27,28). Although circulating TRAIL levels are elevated in human viral hepatitis, a conclusive role in mediating apoptosis has not been established (29). Therefore, TRAIL holds the potential for enhancing therapeutic apoptosis of malignant or virally infected cells without collateral damage.

Lysosomes

Lysosomes are enzyme-filled, membrane-lined or ganelles that along with peroxisomes form the acid vesicle system. The intraorganelle pH of lysosomes is acidic, and lysosomal enzymes optimally function at this acidic pH. Abnormal lysosomal morphology is found in acute and chronic liver diseases. The accumulation of phospholipids in lysosomes (phospholipidosis) is associated with drug-induced liver injury by amiodarone, trimethoprin–sulfamethoxazole, alcohol, and ketoconazole. Lipid accumulation in lysosomes is also a feature of hepatocyte steatosis. Iron, copper, and lanthane are also lysomotropic and toxic to lysosomes.

The extent of release of lysosomal contents (permeabilization) leads to either necrotic cell death, if exuberant, or apoptotic cell death, if the release is controlled. Lysosomal permeabilization may simply involve accumulation of lysomotropic agents with subsequent disruption and release of their content into the cytoplasm. Additionally, reactive oxygen species (ROS), toxic bile salts, sphingosine, free fatty acids (FFAs), ceramide, and TNF-α lead to selective lysosomal permeabilization with subsequent apoptotic cell death. Lysosomal permeabilization leads to the release of lysosomal enzymes into the cytosol, which trigger the mitochondrial pathway of cell death (30,31,32).

Endoplasmic reticulum

The ER is a network of intracellular membranes, the largest membranous organelle in a cell. Its primary functions are to synthesize lipids and proteins. Perturbations that interfere with ER function have been collectively called *ER stressors*. The ER stress pathway is activated by misfolded proteins, glycosylation inhibitors, glucose deprivation, altered glycosylations, ultraviolet (UV) irradiation,

oxidative stress, and alterations in intracellular calcium level. These stimuli lead to accumulation of abnormal proteins, the so-called unfolded protein response (UPR), which is the hallmark of ER stress (33). Sustained ER stress leads to cell death through at least two well-described mediators. Interactions between regulatory ER-localized kinases (e.g., IRE1, PERK, and ATF6) and downstream effectors lead to activation of c-jun *N*-terminal kinase (JNK) (34). JNK then activates the mitochondrial pathway of apoptosis (35,36). Another pathway involves the transcription factor CHOP (c/ebp

homologous protein). The downstream effectors of CHOP are not well defined, but the factor regulates apoptosis transcriptionally and nontranscriptionally through protein–protein interactions (37,38). ER stress is best described in α_1-antitrypsin (α_1-AT) deficiency in which abnormal protein is aggregated in the ER with resultant downstream death signaling (39).

Oxidative stress

Oxygen and oxidative reactions are an essential part of aerobic oxidative phosphorylation, the process that converts nutrient energy into adenosine-5′-triphosphate (ATP), the currency of cellular energy. Oxidative stress is a consequence of this aerobic metabolism. The generation of ROS, (e.g., $O_2^{\cdot-}$, H_2O_2, OH·) and subsequent formation of oxidative products of amino acids, proteins, carbohydrates, lipids, and DNA constitutes oxidative stress. The ROS superoxide ($O_2^{\cdot-}$) also interacts with nitric oxide (NO) to form the reactive nitrogen species, peroxynitrite ($ONOO^-$). Peroxynitrite is a potent oxidizing and nitrating agent. It can nitrate tyrosine residues in several cellular proteins and iron residues in metalloproteins. Given the dependence on aerobic metabolism for energy, cells harbor several antioxidant defense systems to protect themselves from oxidative and nitrative stress. When antioxidant defense systems are overwhelmed, oxidative and nitrative damage ensues. In acute injury models ROS are associated with mitochondrial permeability transition (MPT) in both apoptotic and necrotic cell death. Lipid peroxidation, oxidized DNA, and nitrated proteins are seen in several models of chronic oxidative stress–inducing liver injury (40,41,42,43). Iron overload, copper overload, chronic ethanol consumption, nonalcoholic steatohepatitis (NASH), and viral hepatitis are all associated with oxidative cellular constituent damage. The importance of oxidative stress is further underscored by the well-established use of the antioxidant *N*-acetylcyteine in acute acetaminophen-induced liver failure.

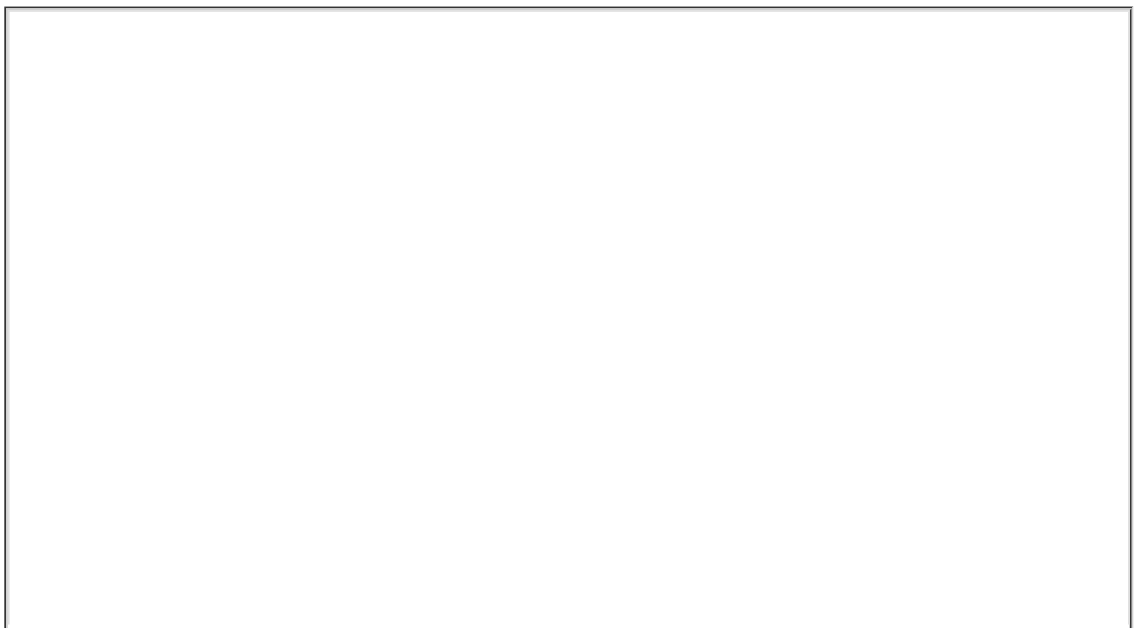
Mitochondria

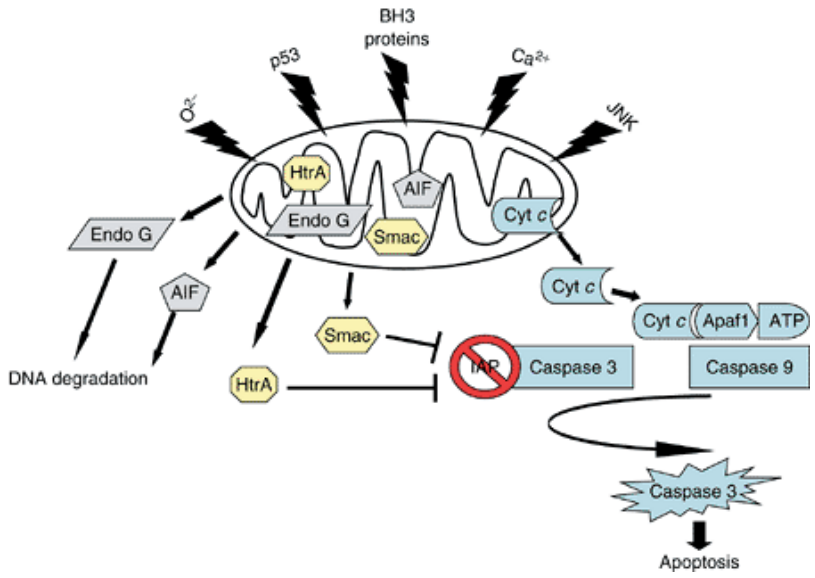
Mitochondria are bound by two membranes, the outer and inner mitochondrial membranes. These two membranes enclose the intermembrane space; the inner membrane is folded into cristae and encloses the mitochondrial matrix. Proapoptotic proteins such as cytochrome *c*, second mitochondrial activator of caspase/direct inhibitors of apoptosis–binding protein with low pI (SMAC/DIABLO), HtrA2/Omi, apoptosis-inducing factor (AIF), and endonuclease G are located within the intermembrane space. The outer mitochondrial membrane is normally impermeable to these proteins, permitting the cell to survive and function. Mitochondrial permeabilization results in the release of these proteins into the cytosol with activation of downstream proteases, culminating in apoptosis (44,45,46) (Fig. 10.5).

Mitochondria are essential for the execution of apoptosis in hepatocytes.

Apoptosis can be divided arbitrarily into three phases, a premitochondrial phase, a mitochondrial phase, and a postmitochondrial phase. The premitochondrial phase is discussed in detail in the section on DRs and other perturbations that culminate in the mitochondria. The end result of the mitochondrial phase is selective mitochondrial permeabilization. This process is regulated by the Bcl-2 family proteins. The Bcl-2 family is divided into pro- and antiapoptotic members. Of the antiapoptotic proteins, Bcl-2, Bcl-XL, and Mcl-1 are important in the liver. The proapoptotic proteins are further divided into multidomain (Bak and Bax) and BH-3 domain only (Bid, Noxa, Puma, Bim, and Bad). Bax is located in healthy individuals in the cytosol but undergoes conformational change and inserts into the mitochondrial membrane to exert its actions. Bak, on the other hand, is an integral mitochondrial protein also activated by conformational change. Bak and Bax then form pores in the outer mitochondrial membrane, leading to permeabilization. The BH-3–only protein Bid is cytosolic and involved in the premitochondrial phase of apoptosis. Its activation is mediated by death receptor–activated caspase 8, and therefore, it serves as a link from the extrinsic to the intrinsic pathway of apoptosis. Bim is activated by its release from cytoskeletal dynein motor complex, and Bad is activated by dephosphorylation. The goal of the proapoptotic members is mitochondrial permeabilization, and the goal of the antiapoptotic members is to prevent just this. The manner in which this occurs is complex and not fully known; it is, however, not just the sum total of the pro- and antiapoptotic signals (47).

Mitochondrial abnormalities, both structural and functional, are associated with liver disorders. Drugs and xenobiotics can inhibit the electron transport chain, uncouple oxidative phosphorylation, impair fatty acid oxidation, damage mitochondrial DNA, and impair mitochondrial DNA repair. Mitochondrial abnormalities in alcohol-induced liver disease are well described (42). Ethanol-driven generation of ROS is associated with oxidative damage to lipids, proteins, and DNA. Moreover, high levels of TNF-α expression, driven by ethanol, also lead to an increase in ROS. Ethanol toxicity, therefore, leads to the MPT that may occur either through DR-mediated pathways or through intrinsic cellular stress. Bile acids, whose levels are characteristically elevated in cholestatic liver diseases, can also trigger mitochondrial dysfunction (48,49,50,51). Similarly, mitochondrial dysfunction is associated with NASH, although the molecular mediators need to be defined (52,53,54).





▪ **Figure 10.5** The role of mitochondria in cell death. Mitochondrial dysfunction is an obligate event in hepatocellular apoptosis. Intrinsic apoptotic stimuli such as p53, calcium, oxidative stress, and c-jun *N*-terminal kinase (JNK), as well as extrinsic apoptotic stimuli (BH-3 proteins), converge on mitochondria. Mitochondrial dysfunction leads to release of several proapoptotic factors. Endonuclease G (Endo G) and apoptosis-inducing factor (AIF) lead to deoxyribonucleic acid (DNA) degradation. Second mitochondrial activator of apoptosis (smac) and HtrA inhibit a set of proteins known as *inhibitors of apoptosis* (*IAP*) leading to the release of caspases 3 and 9. Cytochrome *c* complexes with Apaf 1 and adenosine-5′-triphosphate (ATP) to form the apoptosome that leads to the activation of caspases 3 and 9 that are released from IAP inhibition.

Necrosis

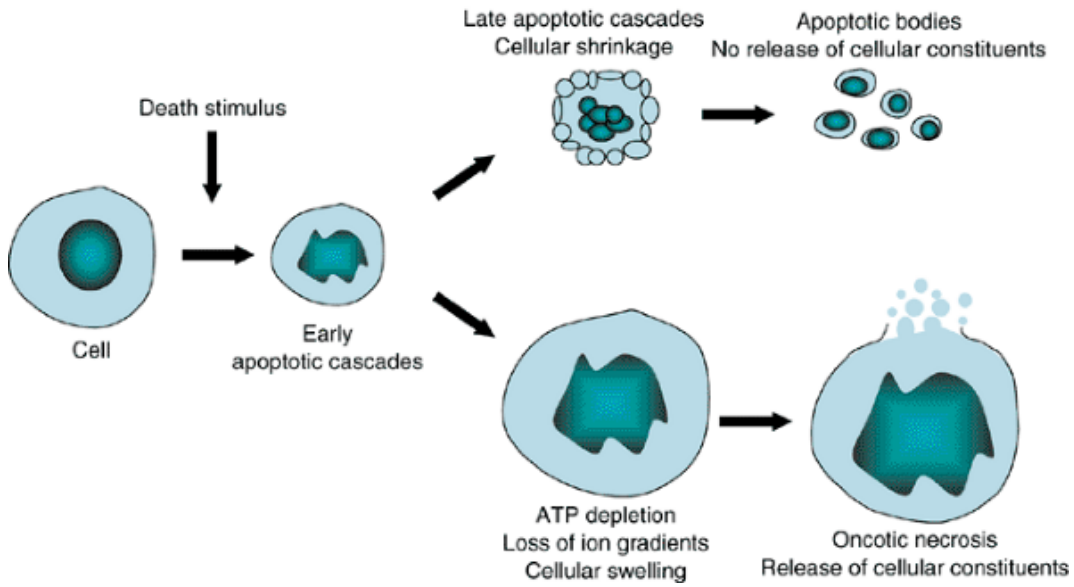
Necrosis has been defined as an energy-independent process, in which cells swell and lyse, releasing their contents. It has been considered "unprogrammed" compared with apoptosis or programmed cell death. The death signaling in necrosis is considered absent or disordered and the cell death equated to the supernova of cellular death phenomenon. Inflammation is viewed as a consequence of phagocytosis of cellular debris and thought to occur more in necrosis because of exuberant release of cellular contents (Fig. 10.6). Although historically viewed as distinct processes, in the last few years, similarities between the two processes have surfaced. Morphologically, hepatocytes with dual characteristics have been observed in vivo in injury models such as ischemia-reperfusion (IR). Regulated, albeit caspase-independent, and death receptor–independent cell death has broadened the definition of necrosis and blurred the erstwhile clear-cut distinction from apoptosis. In addition Fas-mediated necrosis has been described (55). Increasingly, necrosis is viewed as a massive yet programmed cell death (56,57).

Cellular ATP depletion activates necrotic cell death. During ischemia a lack of

oxygen and nutrients leads to a dramatic and absolute inability to generate ATP. This activates processes leading to cellular destruction and necrosis. Exposure to massive ischemia, nitrative/oxidative stress, and xenobiotics can all result in hepatic necrosis (43,58). In some instances the magnitude of noxious stimulus controls the subsequent mode of cell death, with apoptosis resulting from a lesser stimulus and necrosis occurring with the greater magnitude of hepatic insult.

Mitochondria in necrosis

In addition to their role in apoptosis, mitochondria mediate necrotic cell death (Fig. 10.7). MPT, an abrupt increase in the permeability of the inner and outer mitochondrial membranes, occurs in necrosis. This is mediated by the MPT pore, (permeability transition pore [PTP]). The PTP is formed by voltage-dependent anion channel (VDAC) on the outer membrane and adenine nucleotide transporter (ANT) on the inner membrane along with cyclophilin D. The permeability transition leads to dissipation of the electrochemical gradient across the inner mitochondrial membrane, uncoupling of oxidative phosphorylation, and an inability to synthesize new ATP. Calcium and mitochondrial proteins are released as well. Mitochondrial swelling occurs secondary to an increase in membrane permeability, leading to mitochondrial rupture and necrotic cell death (59).



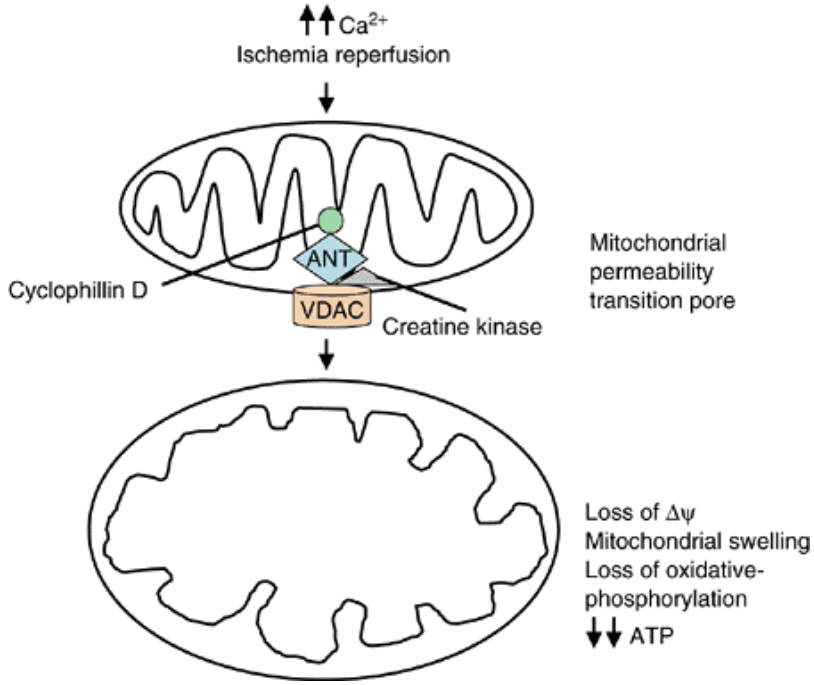
▪ **Figure 10.6** Apoptosis and necrosis are divergent endpoints of common initiating signals. Death-initiating signals lead to activation of early apoptotic signals. In the milieu of adenosine triphosphate (ATP) depletion, loss of ion gradients and cellular swelling ensues. This results in mitochondrial and cellular rupture with release of intracellular content. In a controlled and energy-replete environment, the apoptotic cascade proceeds, leading to nuclear condensation and fragmentation of cells into apoptotic bodies. Physiologically, these apoptotic bodies are engulfed and efficiently removed by Kupffer cells.

Disease Mechanisms

Alcohol-Related Liver Disease

Alcoholic steatohepatitis (ASH) is characterized by steatosis, hepatocyte apoptosis, and acute inflammation. The cellular mechanisms and cytokine milieu leading to alcohol-induced liver injury are well defined. The factors that impart each individual's susceptibility to liver damage are less well understood. In experimental models, ethanol induces changes in mitochondrial and microsomal function with subsequent apoptosis and necrosis (60). Oxidative stress occurs with acute and chronic ethanol ingestion. MPT occurs as a result of oxidative stress, leading to the release of cytochrome *c* and other mitochondrial enzymes,

activation of effector caspases, and apoptosis (61,62). Neutralization of ROS with antioxidants, inhibition of MPT, or inhibition of caspases prevents acute ethanol-induced apoptosis. Oxidative stress also leads to translocation of Bax from cytosol to mitochondria, resulting in mitochondrial dysfunction (63). Induction of cytochrome P-450 2E1 (CYP2E1), a well-known effect of ethanol ingestion, also promotes generation of ROS and may explain how ethanol induces its own toxicity in a feedback loop (60,64). Kupffer cells demonstrate increased expression of CYP2E1 and oxidative stress, and more importantly, they become activated in acute alcohol-mediated liver injury. Once activated, they secrete a number of cytokines, including TNF-α, IL-6, and transforming growth factor β_1 (TGF-β_1) (65,66,67). The role of these inflammatory cytokines is underscored by studies using endotoxinemia to promote TNF-α expression and liver injury and, on the other hand, selective gut decontamination (with antibiotics to reduce portal vein endotoxin levels) with attenuation of both TNF-α signaling and liver injury (65,68). Furthermore, genetic studies in mice demonstrated that TNFR1 is essential for alcohol-induced liver injury (69).



▪ **Figure 10.7** The mitochondrial permeability transition (MPT) pore.

Mitochondria mediate necrotic cell death in the liver. The mitochondrial permeability transition pore (PTP) is formed by voltage-dependent anion channel (VDAC) on the outer membrane and adenine nucleotide transporter (ANT) on the inner membrane along with cyclophilin D. Opening of the PTP leads to mitochondrial swelling, rupture, and release of intermembrane proteins.

Apoptosis occurs in patients with ASH and is correlated with bilirubin, aspartate aminotransferase (AST), and grade 4 steatohepatitis (70,71,72). Hepatic Fas receptor expression is enhanced in ASH, compared with normal livers (70). Studies on sera of patients with ASH have shown increased circulating levels of Fas, FasL, and TNF-α. TNF-α levels correlate with mortality in these patients (73,74,75,76). Hepatocyte apoptosis also correlates with Maddrey's score, a prognostic indicator in acute alcoholic hepatitis. The characteristic inflammatory response occurs secondary to hepatocyte apoptosis and also because of the direct effects of ethanol on Kupffer cells, leading to cytokine production (66). In summary, alcohol-induced liver injury occurs in the setting of oxidative stress and a proinflammatory cytokine environment that together induce hepatocyte apoptosis and consequent inflammation. Apoptosis correlates with the severity of liver injury. Inhibition of the apoptotic signaling pathway holds the potential for future therapies of alcoholic liver disease.

Nonalcoholic Steatohepatitis

Nonalcoholic fatty liver disease (NAFLD), the hepatic component of the metabolic syndrome, has become the most common liver disorder in the United States (77). Hepatocyte apoptosis is a prominent feature of NASH and correlates with disease severity, disease progression, and fibrosis (78). This association underscores the importance of hepatocyte apoptosis and raises the question why only certain steatotic cells die. Recent mechanistic understanding of hepatocyte apoptosis in NASH have elucidated the role of DR ligands, circulating and intrahepatic FFAs, inflammatory cytokines, mitochondrial abnormalities, and genes of fat regulation (53,78,79).

Early pathogenetic studies have described increased Fas and TNFR1 expression in livers of patients with NASH (1). Furthermore, circulating TNF-α levels are also elevated in patients with NASH. This is confirmed by studies in animal models of steatosis, in which apoptosis and inflammation are enhanced after administration of DR ligands, Fas, and TNF-α (80). These data demonstrate the sensitivity of the steatotic hepatocyte to a secondary insult. Insulin resistance, a feature of obesity and the metabolic syndrome, leads to elevated plasma FFA levels. Recent advances have been made in elucidating the cellular mechanisms by which FFA leads to apoptosis in the steatotic hepatocyte, a process termed *lipoapoptosis*. At least two mechanisms have been described. Lysosomal permeabilization by FFA in an in vitro model using HepG2 cells has partially elucidated the role of the intrinsic apoptotic pathway. Indeed, this has been confirmed in patients with NASH who show evidence of lysosomal permeabilization and release of cathepsin B (79). Furthermore, TNF-α expression occurs downstream of, and is partially dependent on, lysosomal permeabilization in this model. A separate in vitro study of the sensitivity of steatotic HepG2 cells to Fas ligand, presumably through upregulation of Fas receptor, provides another piece of the puzzle that integrates

the well-characterized death ligand sensitivity of steatotic livers with the primary metabolic abnormality observed in this syndrome (80). Mitochondria are central to cell death, and FFA-mediated mitochondrial dysfunction in HepG2 cells, as well as primary mouse hepatocytes, has been described as well. One way in which mitochondrial dysfunction can be activated is by a family of signaling enzymes, the mitogen-activated protein kinases (MAPKs). FFA-induced activation of c-JNK, a proapoptotic MAPK, with subsequent JNK-dependent apoptosis in hepatocytes is also an important pathway of FFA-induced lipoapoptosis. Mitochondrial dysfunction in this model results from upregulation of proapoptotic Bcl-2 family proteins (Malhi H, Gores GJ, *unpublished observations*, 2006).

Abnormal mitochondrial structure and function also occur in patients with NASH (53). Megamitochondria with crystalline inclusions, decreased hepatic mitochondrial DNA content, and decreased respiratory chain function occur. CYP2E1 level is increased in patients with NASH, and there is some evidence for increased oxidative stress, which may be stimulated by enhanced FFA-driven mitochondrial β-oxidation. Therefore, the role of mitochondrial abnormalities and oxidative stress as activators of the intrinsic pathway of apoptosis needs to be studied further.

Viral Hepatitis

Hepatitis B virus (HBV) and hepatitis C virus (HCV) cause both acute and chronic hepatocellular infection. In acute viral infection, immune-mediated apoptosis leads to elimination of infected cells, and for chronic infection to be successful, virally infected hepatocytes must evade apoptosis (81). The cell injury in acute infection occurs in two phases. The first phase in acute HBV and HCV infection involves CTL-induced Fas-mediated hepatocyte apoptosis (82,83). The second wave of injury is triggered by apoptosis and occurs as a nonspecific necroinflammatory response that also damages bystander cells that do not express viral antigens (84,85). Also, the virus has a small direct cytopathic effect.

In chronic infection there is ongoing, low-grade, Fas-mediated apoptosis. Apoptosis correlates with histologic severity of chronic hepatitis (86). In sera from patients with chronic HBV and HCV infection sFas levels are increased and correlate with alanine aminotransferase (ALT) levels, histology, and response to therapy (87,88). Furthermore, at the onset of treatment with interferon, sFas levels increase in parallel with ALT values, suggesting enhanced Fas-mediated immune clearance of infected cells (89). Active alcohol consumption in hepatitis C leads to a significant increase in hepatocyte apoptosis that correlates with increased Fas levels, pointing toward a convergence of two distinct apoptotic stimuli on the Fas signaling pathway (90). Not only is there evidence of apoptosis but also soluble markers of apoptosis hold the promise of surrogacy, decreasing the need for repeated liver biopsies (91). It is also clear from several experimental models that HCV proteins regulate apoptosis (92). HCV core protein confers sensitivity to TRAIL-mediated apoptosis in cells previously resistant to its effect (93). Other HCV proteins such as NS3 can activate caspase 8–mediated apoptosis, independent of Fas (94). The inhibition of apoptosis of virally infected hepatocytes possibly provides a mechanism for both viral persistence and development of hepatocellular carcinoma (HCC). Indeed, in a mouse model of hepatic carcinogenesis, the introduction of core E1 and E2 proteins lead to the formation of larger tumors (95,96,97,98,99). In complementary in vitro

experiments, HCV core protein inhibited Fas- and TNF-α-mediated apoptosis in HepG2 cells (100).

Similarly, in chronic HBV infection both Fas receptor and TNFR1 expression are increased in hepatocytes (101). Levels of circulating Fas and TNF-α are increased as well and correlate with severity of infection (88,102). HBV X protein (Hbx) has complex biologic functions in the host, which remain controversial and are reported to attenuate and promote cell death (103,104,105,106,107,108). Therefore, both HCV and HBV infection, although cleared by immune-mediated hepatocyte apoptosis, regulate the apoptotic machinery to establish chronic infections predisposing to hepatocarcinogenesis.

Ischemia-Reperfusion Injury

IR injury occurs during liver transplantation, liver surgery, and hypotensive states. Hemodynamic changes are an integral part of liver transplantation surgery, therefore, understanding the mechanisms of cold ischemia (CI)/warm reperfusion (WR) injury should promote therapeutic strategies to minimize injury and improve allograft function. WR occurs in other forms of liver surgery and hypotensive states. SECs are the immediate target of CI/WR injury, with hepatocyte injury occurring after prolonged periods of ischemia. In contrast, hepatocytes are the primary target in WR injury. Cold storage alone leads to apoptosis of SEC. Use of caspase inhibitors significantly decreases SEC apoptosis and improves survival after orthotopic liver transplantation (OLT) (109). Kupffer cells are activated and secrete numerous cytokines that in turn activate apoptosis and attract inflammatory cells. Depletion of Kupffer cells using gadolinium chloride decreases SEC apoptosis and liver injury in CI/WR (110,111).

IR injury involves hepatocyte apoptosis, which also correlates with the duration of ischemia and the presence of preexisting liver damage (112). Activation of NFκB, TNF-α, and Fas modulate IR-induced hepatocyte apoptosis (113,114). NFκB has a biphasic activation after IR; the initial phase promotes expression of TNF-α, leading to apoptosis and inflammation, and the later phase is protective, such that selective inhibition of activation of the later phase enhances liver injury, and nonspecific inhibition attenuates injury (115). Historically, necrosis and apoptosis both have been thought to mediate IR injury; recent evidence, however, points toward apoptosis as the principal mode of hepatocyte cell death in IR (58,116). In experimental models TNF-α-dependent apoptosis, capsase-dependent hepatocyte apoptosis, and increase in levels of FasL expression were observed (113,117). Stress-activated protein kinases, such as JNK, are also activated soon after OLT (118). Use of JNK inhibitors preserves hepatic architecture and attenuates injury (119). Expression of Bcl-2, an antiapoptotic protein, by several different modalities, protects hepatocytes against ischemic apoptosis and liver injury (120). The use of small interfering ribonucleic acid (siRNA) to decrease the expression of caspases 8 and 3 also reduces IR injury. In summary, IR injury is mediated by apoptosis of both parenchymal and nonparenchymal liver cells. Inhibition of apoptosis in experimental studies has improved the outcomes of OLT; this offers promising interventions to maximize allograft function.

Cholestatic Injury

Cholestasis, an impairment in bile flow and/or secretion, is characterized by an increase in hepatocellular bile acid concentrations. At a cellular level, the effects of hydrophobic bile acids are well understood. Glycine-conjugated

chenodeoxycholic acid (GCDC) is more toxic than taurine-conjugated chenodeoxycholic acid (TCDC) (121,122). Toxic bile acids induce hepatocyte apoptosis in vitro and also in vivo in animal models of extrahepatic cholestasis (bile duct–ligated animal). There is evidence of involvement of the death ligands, Fas and TRAIL, in bile acid–mediated apoptosis (123,124). The importance of Fas receptor in this pathway is proved further by studies in mice deficient in Fas receptor (*lpr*). Following bile duct ligation in these *lpr* mice, hepatocyte apoptosis is attenuated (125). Furthermore, in long-term follow-up in these animals, fibrosis is attenuated as well. This study underscores the importance of the paradigm that hepatocyte apoptosis acts as a fibrogenic stimulus, resulting ultimately in liver cirrhosis.

Fas-induced apoptosis is not the only mechanism of hydrophobic bile acid toxicity. In cholestatic *lpr* mice, hepatocyte apoptosis eventually occurs, although delayed and attenuated when compared to wild type mice. Bax levels and translocation to mitochondria are increased in cholestatic *lpr* mice and explains the onset of apoptosis (5). Inhibition of apoptosis by inhibiting Bid prevents both Fas-dependent and Fas-independent bile acid–induced hepatocyte apoptosis (126). Furthermore, in Fas-deficient cells, the role of TRAIL-R2 in bile acid–induced hepatocyte apoptosis has been unmasked. TRAIL activation by GCDC leads to the recruitment of the classical TRAIL–death-inducing signaling complex (DISC), with activation of caspases 8 and 10, involvement of mitochondria, release of cytochrome *c*, and apoptosis (49).

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are the two commonest etiologies of adult intrahepatic cholestasis. Immune-mediated apoptosis of biliary epithelial cells (BECs) is well defined in PBC (127,128). Pyruvate dehydrogenase complex (PDC), a mitochondrial protein, is expressed on the cell surface membrane of BEC in PBC. Autoantibodies and autoreactive T cells exist against this antigen, perpetuating immune-mediated BEC apoptosis. PDC is normally sequestered in the inner mitochondrial membrane. Although the perpetuation of autoimmune injury in response to PDC is understood, the initial apoptotic stimulus that leads to mitochondrial dysfunction and expression of PDC on BEC surface is unknown. In experimental models it was found that immunoreactive PDC migrated from the mitochondria to the plasma membrane of cells after the induction of apoptosis (3). Therefore, apoptosis plays a role in bile duct injury and in the ensuing hepatocellular injury seen in cholestasis.

Wilson Disease and Hemochromatosis

Wilson disease is characterized by a hepatocellular defect resulting from mutations in a copper-transporting P-type ATPase (ATP7B) with an inability to excrete copper in bile. This leads to copper accumulation in hepatocytes, which is cytotoxic (129). The Long-Evans Cinnamon (LEC) rat is a spontaneous mutant that mimics human Wilson disease (130). This animal shows evidence of chronic oxidative damage, such as lipid peroxidation and DNA strand breaks (131,132). This suggests a role for oxidative stress because copper has redox activity, such as the Fenton and Haber-Weiss reactions, leading to the generation of free radicals and oxidative damage to lipids, proteins, and DNA. In vitro data shows that copper overload also leads to p53-dependent cell death (133).

The course of Wilson disease in humans runs the gamut from FHF to mild chronic hepatitis. In patients with Wilson disease who have FHF, high levels of apoptosis, Fas receptor, and Fas messenger RNA (mRNA) were detected (134). Oxidatively damaged and bulky DNA, indicative of damaged DNA with adduct formation, was

detected in patients with Wilson disease (40,135). Therefore, copper overload causes apoptotic hepatocellular death in patients with Wilson disease.

Oxidative stress is a direct consequence of iron overload because of the redox activity of iron and the generation of oxygen free radicals. As with copper excess, the generation of ROS occurs through Fenton and Haber-Weiss reactions. Oxidative damage to all cellular constituents ensues and, indeed, oxidative DNA adducts are found in patients with hereditary hemochromatosis (41). Although iron is not a direct carcinogen, iron overload is associated with increased risk for the development of HCC (136).

α_1-Antitrypsin Deficiency

α_1-AT is normally secreted predominantly by hepatocytes. Patients with α_1-AT deficiency produce an abnormal variant of the protein, resulting in a failure of hepatocytes to secrete it into the serum. This leads to accumulation of the abnormal protein within hepatocytes. The normal homeostatic response leads to enhanced degradation of the abnormal protein, but in individuals unable to breakdown the accumulated protein, hepatocyte injury ensues. The ER is a major site of degradation of abnormal proteins in the body. Briefly, the accumulation of abnormal α_1-AT in the ER leads to

activation of autophagic processes. Mitochondrial dysfunction and caspase 3 activation occur downstream of this ER stress response (39). The molecular pathways that mediate α_1-AT-induced ER stress and mitochondrial dysfunction are not well defined and are an area for future research.

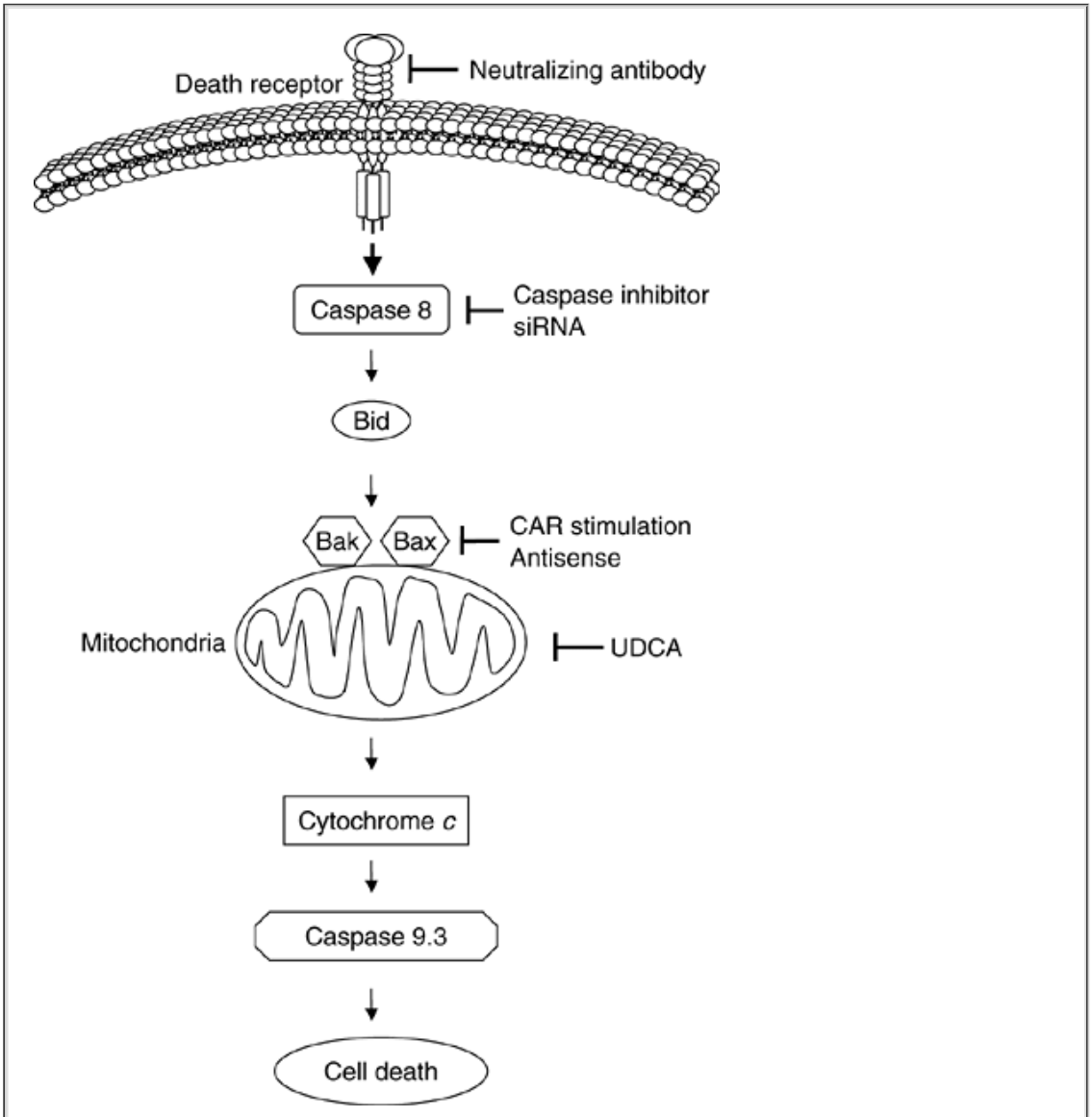
Fulminant Hepatic Failure

Several lines of experimental data and human observations point to the importance of DR-mediated apoptosis in FHF. In a seminal paper Lacronique et al. (137) it was reported that induced massive hepatocyte apoptosis and FHF in mice using an anti-Fas antibody activated the death signaling cascade and rescued livers from apoptosis and animals from death by increasing the expression of antiapoptotic Bcl-2 (137,138). In patients with FHF, levels of both Fas receptor and Fas ligand expression in hepatocytes are high. Fas ligand levels are elevated in infiltrating lymphocytes, circulating lymphocytes, and sera of patients as well. Hepatocyte apoptosis in addition to enhanced Fas expression has been observed in FHF of different etiologies (134,139,140). In addition to Fas, circulating levels of TNF-α and TNF-α receptors are increased in patients with FHF and correlate with the recovery of native liver function (141). Besides activation of NFκB to aid in recovery, the dichotomous role of TNF-α is further developed in FHF, in which it increases the expression of FADD protein, perhaps augmenting Fas sensitivity. In summary, FHF is accompanied by several cytokine changes, of which Fas clearly mediates apoptosis. TNF-α and other cytokines serve dichotomous roles, promoting apoptosis, inflammation, and recovery. Enhancing the milieu in favor of recovery by inhibiting Fas-mediated apoptosis appears to be a promising therapeutic strategy, one that should be developed further with human clinical trials, given the shortage of donor organs.

Therapeutic Implications

Understanding apoptotic cascades in liver disease has unraveled novel therapeutic opportunities (Fig. 10.8). Neutralization of TNF-α in treatment of alcoholic hepatitis has shown early promise (142,143). Ribonucleic acid

interference (RNAi) therapy selectively manipulates a cell's genetic machinery to reduce expression of the protein of interest. Disruption of Fas and caspase 8 gene expression, using RNAi, ameliorated injury and improved survival in experimental FHF and immune-mediated hepatitis (140,144). Increased expression of antiapoptotic Bcl-2 and caspase inhibition protected against FHF (137,145). Similarly, targeted hepatic delivery of NCX-1000, a NO conjugate of urosdeoxycholic acid (UDCA), that selectively releases NO in the liver even after onset of apoptosis protects from acetaminophen (*N*-acetyl-p-aminophenol [APAP])-induced FHF (146). In a model of IR injury, silencing initiator and effector caspases had a salutary effect on the liver (116). UDCA has a dual effect of preventing mitochondrial permeabilization and apoptosis induced by bile acid, ethanol, Fas, and TGF-β_1 (147) and also promoting the activation of survival signals in cells (148). Furthermore, in animal studies an antiapoptotic molecule, IDN-6556 (a pan-caspase inhibitor), reduces cholestatic hepatocyte apoptosis, stellate cell activation, liver injury, and resultant fibrosis (149). At a mitochondrial level, the disruption of proapoptotic Bcl-2 family proteins also prevents bile acid–induced apoptosis (126).



▪ **Figure 10.8** Therapeutic targets in apoptosis. Schematic representation of

potential therapeutic targets in liver diseases. Apoptosis can be inhibited at several levels. Death receptor and ligand interaction can be prevented by neutralizing antibodies. Pharmacologic inhibitors, small interfering ribonucleic acid (siRNA), antisense molecules, modulation of Bcl-2 family pro- and antiapoptotic proteins with nuclear receptor agonists, for example, constitutive androstane receptor (CAR), are all potential therapeutic strategies. Ursodeoxycholic acid (UDCA) has been in clinical use for cholestatic liver disease.

The fact that apoptosis can be modulated temporally after cells have been exposed to apoptotic stimuli is appealing in its applicability to the clinical scenario. Indeed, several studies in experimental models of acute and chronic liver disease have demonstrated amelioration of liver apoptosis and injury and improved survival, with inhibition of apoptosis even after exposure to the apoptogenic stimulus. Future studies should be directed toward development of rational antiapoptotic therapies targeted to the liver.

Acknowledgments

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Annotated References

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Apoptosis and necrosis have classically been viewed as different modes of cell death, with opposite physiologic and pathologic effects. This article explores the gray areas in this erstwhile black or white delineation.

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This is a comprehensive review of death receptors and their signaling pathways. It is essential reading for anyone wishing to understand death receptor–mediated signals.

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The importance of apoptosis, death receptor signaling, and activation of inflammatory pathways in two common and relevant liver diseases are described.

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Chapter 11
The Liver in Systemic Disease

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Key Concepts

- Liver disease is highly prevalent in patients with type 2 diabetes mellitus and can range from mild steatosis to cirrhosis and hepatocellular carcinoma. The impact of liver disease on mortality in patients with diabetes is substantial.
- Patients with thyroid disorders, especially hyperthyroidism, commonly have abnormal liver chemistries. Thyrotoxicosis can lead to ischemic hepatic injury, whereas hepatic dysfunction in myxedema may result from right-sided heart failure. Medications used to treat hyperthyroidism can lead to hepatotoxicity.
- Celiac disease may account for otherwise unexplained liver chemistry abnormalities in a substantial number of affected persons.
- Liver disease associated with various rheumatic disorders is commonly the result of the medications used to treat these conditions.
- Liver dysfunction in patients with sickle cell disease results from red cell sickling within hepatic sinusoids and must be distinguished from cholecystitis and choledocholithiasis.
- Hepatic involvement is common in amyloidosis and sarcoidosis. In amyloidosis, hepatic involvement rarely causes clinical manifestations but indicates an ominous prognosis. Hepatic sarcoidosis may occasionally be complicated by cirrhosis and portal hypertension.
- Involvement of the liver by lymphoma is more common in patients with non-Hodgkin's lymphoma than in those with Hodgkin's disease. Rarely, hepatic lymphoma can result in acute liver failure.

The liver interacts in multiple ways with other organ systems and is often involved by systemic disease processes. This chapter reviews the systemic disorders that most frequently involve the liver and includes only those diseases that are not covered in detail in other chapters.

Endocrine Disorders

Diabetes Mellitus

Approximately 17 million people in the United States are affected by type 2 diabetes mellitus, and liver disease is one of the leading causes of death in this patient population (1,2). In fact, the standardized mortality rate for liver disease among patients with type 2 diabetes mellitus is higher than that for cardiovascular disease. Liver disease in patients with type 2 diabetes mellitus can range from nonalcoholic fatty liver disease (NAFLD) to cirrhosis and hepatocellular carcinoma (HCC) (1,3). In addition, evidence suggests that the frequency of chronic hepatitis C is increased among patients with type 2 diabetes mellitus (4).

The most common liver disease associated with diabetes mellitus is NAFLD (1,5,6) (see Chapter 39). It has been estimated that up to 70% of patients with type 2 diabetes mellitus have some form of NAFLD,

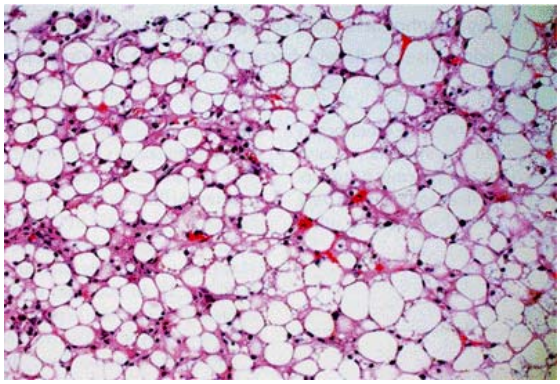
and this rate may reach 100% in patients with both diabetes and obesity (7). Nonalcoholic steatohepatitis (NASH) is almost three times as frequent in persons with type 2 diabetes mellitus as in persons without type 2 diabetes mellitus and is estimated to be present in up to 50% of patients with type 2 diabetes mellitus and NAFLD. Even more alarming is the fact that up to 19% of patients with type 2 diabetes mellitus and NAFLD have cirrhosis (1).

Patients with diabetes and NAFLD typically present with asymptomatic elevations in serum aminotransferase levels; massive hepatomegaly has been described in rare cases. Mild elevations in serum alkaline phosphatase and γ-glutamyl transpeptidase levels have also been described.

Fatty infiltration of the liver in patients with type 2 diabetes mellitus is typically diffuse (Fig. 11.1), although fat accumulation may be focal (1). Steatosis can generally be diagnosed by abdominal ultrasonography, which reveals a diffuse increase in hepatic echogenicity, with focal areas of fat sparing that appear as mass lesions (1,8). Although magnetic resonance spectroscopy can quantify hepatic steatosis, liver biopsy is

required for the detection of NASH and fibrosis.

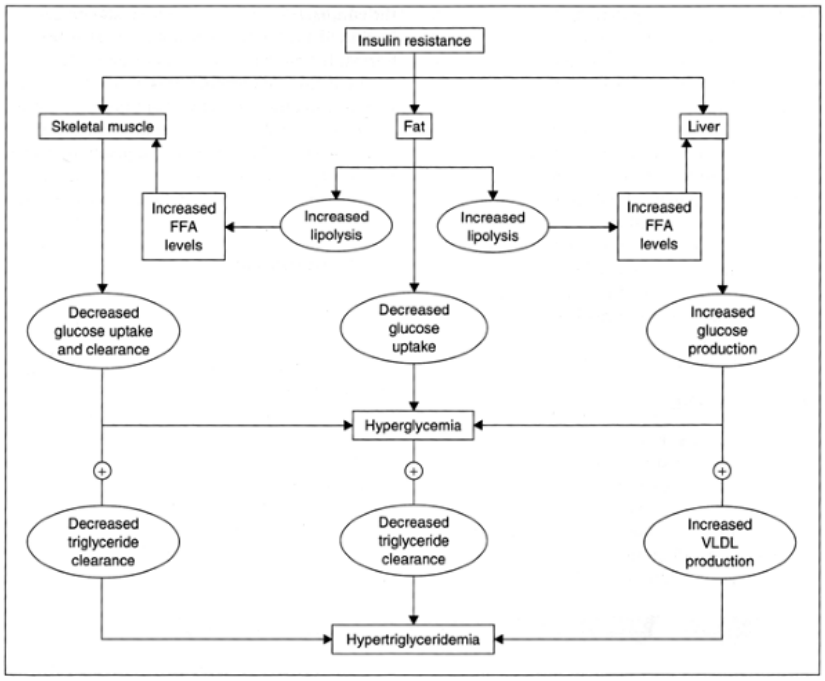
In addition to steatosis, liver biopsy findings in patients with NASH include various combinations of inflammation, Mallory's hyaline, balloon degeneration of hepatocytes, and fibrosis. Nuclear glycogenation, producing a vacuolated appearance of hepatocyte nuclei on light microscopy, may be found in up to 75% of patients with type 2 diabetes mellitus. This feature is not pathognomonic of diabetes and may also occur with bacterial inflammation, tuberculosis, cirrhosis, hepatitis, and Wilson disease, and even in normal livers (1).



▪ **Figure 11.1** Liver biopsy specimen demonstrating severe steatosis in a patient with diabetes mellitus and obesity (hematoxylin and eosin). (Courtesy of John Hart, MD, Chicago, IL: Department of Pathology, University of Chicago Pritzker School of Medicine.)

Insulin resistance and obesity appear to be the common factors that link NAFLD to diabetes mellitus (7,9,10) (Fig. 11.2). The resistance to insulin that characterizes type 2 diabetes mellitus leads to decreased peripheral glucose uptake and enhanced adipocyte lipolysis, in turn leading to increased insulin resistance and further deposition of fat in the liver. The biochemical basis of hepatic lipid deposition in patients with type 2 diabetes mellitus remains to be elucidated. One theory postulates that high circulating levels of insulin lead to defective fatty acid oxidation in the mitochondria, thereby resulting in the intracytoplasmic accumulation of triglycerides within the hepatocytes. An inflammatory response may ensue and result in NASH and, in some cases, hepatic fibrosis and, ultimately, cirrhosis (1). As compared with patients without diabetes who have steatosis, patients with type 2 diabetes mellitus having steatosis may be at increased risk for NASH, likely because of the persistence of insulin resistance (1).

The cornerstones of management of patients with diabetes mellitus and hepatic steatosis are metabolic control and weight loss. Although weight loss is clearly important, the ideal rate of weight reduction in patients with hepatic steatosis is debated. Rapid weight reduction can lead to increased formation and hepatic deposition of free fatty acids. The use of pharmacologic agents to reduce steatosis has been investigated (11). Medications such as pioglitazone, rosiglitazone, and metformin that reduce insulin resistance have been shown to improve liver histopathology, hepatic lipid content, and liver biochemical test results in patients with hepatic steatosis and NASH (12,13,14). Although promising, the use of these agents to treat hepatic steatosis and NASH remains investigational.



▪ **Figure 11.2** Metabolic effects of insulin resistance in skeletal muscle, fat, and liver. The resistance to insulin leads to decreased peripheral glucose uptake and enhanced adipocyte lipolysis, in turn leading to increased insulin resistance and further hepatic fat deposition. FFA, free fatty acid; VLDL, very low density lipoprotein. (From Tolman KG, Fonseca V, Tan MH, et al. Narrative review: hepatobiliary disease in type 2 diabetes mellitus. *Ann Intern Med* 2004;141:946–956. Reprinted with permission.)

In addition to the increased frequency of NAFLD in patients with diabetes mellitus, several epidemiologic studies have suggested that the risk of HCC is increased in patients with diabetes mellitus (1,15,16,17,18,19,20,21,22). Studies from Europe and the United States suggest that the risk of HCC may be increased up to fourfold in patients with diabetes mellitus as compared with subjects without diabetes (16,17,18,19,20,21,22). This risk persists even when patients with viral hepatitis are excluded from analysis, thereby suggesting that diabetes may be an independent risk factor for the development of HCC. Cirrhosis resulting from progressive NASH likely provides the background for the development of HCC among patients with type 2 diabetes mellitus. A proposed mechanism of carcinogenesis in this setting includes the formation of reactive oxygen species in hepatocytes as a result of insulin resistance, enhanced lipolysis, and lipid accumulation within hepatocytes (1).

Data from several epidemiologic studies suggest that the frequency of hepatitis C virus (HCV) infection is higher in patients with diabetes mellitus than in the general population, and some evidence even suggests that HCV may have a causative role in the development of diabetes (1,23,24,25,26,27) (see Chapter 30). Several studies have demonstrated a higher frequency of diabetes among patients with HCV infection than among those infected with hepatitis B virus (HBV). In addition, patients infected with HCV who have undergone liver transplantation have a higher frequency of diabetes than do patients who have undergone transplantation for other diseases. Finally, glucose intolerance improves after eradication of HCV with interferon-based therapy. Together, these data suggest that HCV plays a role in the pathogenesis of diabetes. Although HCV genotype 1 is the most common genotype in patients with type 2 diabetes mellitus and HCV infection, the frequency of HCV genotype 2 is disproportionately represented among patients with type 2 diabetes mellitus and HCV infection as compared with those without diabetes (1). The presence of fatty liver is actually highest among patients infected with HCV genotype 3; treatment with interferon-based therapy has been found to reduce the

degree of steatosis in this population (1). Patients with HCV infection and fatty liver are less likely to respond to antiviral therapy than are those without fatty liver (1).

Patients with diabetes have a two- to threefold increased frequency of cholelithiasis as compared with persons without diabetes. Although comorbid obesity may be a contributing pathogenic factor, diabetes alone has been found to be an independent risk factor for the development of gallstones. The mechanism remains speculative, although sluggish bile flow, perhaps caused by diabetic autonomic neuropathy, has been proposed (1,28). A noncirrhotic form of hepatic sinusoidal fibrosis, termed *diabetic hepatosclerosis* and attributed to microangiopathy, has been described in patients with longstanding diabetes mellitus and

microvascular complications (28).

Thyroid Disease

The liver is the major site of thyroid hormone metabolism. Up to 85% of extrathyroidal deiodination of thyroxine (T_4) to triiodothyronine (T_3) and reverse T_3 (rT_3) occurs in the liver. Moreover, plasma-binding proteins of thyroid hormone, including thyroxine-binding globulin, prealbumin, and albumin, are produced by the liver. In addition, the liver is involved in the conjugation, biliary excretion, and oxidative deamination of T_4. Thyroid hormones are also important for normal hepatic function and bilirubin metabolism; for example, thyroid hormone appears to play an important role in regulating the activity of uridine 5′-diphosphate glucuronyltransferase, the hepatic enzyme that is primarily responsible for the conjugation of bilirubin before hepatic excretion (29,30). A summary of the features of thyroid-related liver disease is shown in Table 11.1.

Table 11.1. Hepatic Abnormalities Associated with Various Thyroid Diseases and Therapies

	Thyrotoxicosis	Hypothyroidism	Propylthiouracil therapy	Methimazole and carbimazole therapy
Aminotransferase elevations	++	+	++	–
Alkaline phosphatase elevation	++	+	–	+
Bilirubin elevation	+	+	–	+
Liver biopsy findings	Lobular inflammation, hepatocyte nuclear changes, Kupffer cell hyperplasia	Centrilobular fibrosis in patients with ascites	Nonspecific hepatocellular injury Hepatocellular necrosis in <1% of patients	Intrahepatic cholestasis
	Rarely, centrilobular necrosis and perivenular fibrosis			
	Nonspecific lobular inflammation, intrahepatocytic cholestasis			
Outcome	Laboratory test result abnormalities and liver biopsy findings are reversible in most patients with restoration of the euthyroid state Rarely, fulminant hepatic failure	Clinical findings and laboratory abnormalities are usually reversible within weeks of initiation of thyroid replacement therapy	Full recovery occurs in most patients with a decrease in dose or cessation of therapy Persistent hepatitis occurs in <1% of patients Rarely, fulminant hepatic failure	Liver test result abnormalities and biopsy findings may persist after therapy is discontinued

–, absent; +, occasional; ++, frequent.

Hyperthyroidism

Elevated serum liver biochemical levels can be seen in 45% to 90% of patients with hyperthyroidism (29). Liver chemistry abnormalities can be highly variable; hypoalbuminemia and increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase have been reported. Mild elevations in serum bilirubin levels can be seen in up to 5% of patients with thyrotoxicosis, but marked elevations are rare. The presence of jaundice should prompt a search for other complications of hyperthyroidism, such as cardiac failure, sepsis, and thyroid storm. Clinically, most patients typically present with self-limited hepatitis, but

massive hepatomegaly and fulminant hepatic failure have been described in patients with severe thyrotoxicosis. In most cases, the hepatic abnormalities reverse after restoration of a euthyroid state (29,31,32).

Hepatic oxygen consumption is increased in patients with thyrotoxicosis, but hepatic blood flow is not increased. As a result, the hepatocytes in the centrilobular area (zone 3) of the hepatic lobule are susceptible to ischemia because zone 3 hepatocytes are farthest away from the hepatic arteriole in zone 1. Ischemic injury to centrilobular hepatocytes probably accounts for most of the liver chemistry abnormalities attributable to hyperthyroidism (see Chapter 41). Conditions often associated with hyperthyroidism, such as congestive heart failure, infection, and malnutrition, likely play a role as well (29,31,32).

Liver biopsy findings in patients with mild hyperthyroidism–related hepatic injury are nonspecific and are more likely to be related to associated high-output cardiac failure or rapid weight loss than to thyrotoxicosis per se (29). Biopsy specimens may reveal lobular inflammation with an infiltrate consisting of polymorphonuclear leukocytes, eosinophils, and lymphocytes; hepatocyte nuclear changes; and Kupffer cell hyperplasia. Some patients may exhibit focal or diffuse centrilobular necrosis and perivenular fibrosis, whereas others may show decreased hepatic glycogen content, steatosis, or vacuolization of hepatocytes.

Liver injury associated with antithyroid therapy

Dose-related elevations in serum AST and ALT levels occur in up to 30% of patients taking propylthiouracil (PTU); peak aminotransferase levels generally occur soon after the initiation of therapy (29,30). The elevated aminotransferase levels typically normalize after a reduction in the dose of PTU. Although aminotransferase levels return to normal in most patients after the discontinuation of PTU, hepatitis may persist in less than 1% of patients, with evidence of hepatocellular necrosis in a liver biopsy specimen. Such an idiosyncratic reaction to PTU typically occurs during the first few months of therapy in women younger than 30 years (33,34). Affected patients usually recover completely after discontinuation of PTU, but fulminant hepatic failure resulting in death or the need for liver transplantation has been reported (35,36,37,38). Patients who experience asymptomatic, mild (less than or equal to two times the normal) elevations in serum aminotransferase levels while taking PTU can continue the drug with close monitoring, but the medication should be stopped immediately if aminotransferase levels rise further. Glucocorticoids have been used to treat patients with severe PTU-related hepatic injury, but controlled studies demonstrating the efficacy of such therapy are lacking (29).

Although less common than PTU-associated hepatotoxicity, cholestatic hepatotoxicity may result from methimazole and carbimazole. Female patients older than 50 seem to be at increased risk. Affected persons typically have elevations in serum bilirubin, alkaline phosphatase, and γ-glutamyl transpeptidase levels. The liver test result abnormalities characteristically occur within 2 to 3 months of the initiation of these agents and may persist up to several months after withdrawal of the drug. Liver biopsy specimens usually reveal intrahepatic cholestasis. Because PTU, methimazole, and carbimazole carry the potential for hepatotoxicity, liver tests should be repeated within 3 months of the initiation of any of these medications (29,30,32).

Hypothyroidism

Hypothyroidism affects every organ system in the body, and the liver is no exception. Decreases in hepatic oxygen consumption and in the production, flow, and excretion of bile have all been demonstrated in

patients with hypothyroidism (29,30,31,32). Clinically, these changes may manifest as cholestatic jaundice. In addition, the reduction in bile salt formation and excretion in patients with hypothyroidism may lead to impaired cholesterol metabolism. The resulting hypercholesterolemia, combined with reduced bile flow and gallbladder hypotonia, leads to an increased risk of gallstone formation in affected persons. Liver biopsy specimens from patients with hypothyroidism-related hepatic dysfunction reveal central congestive fibrosis, which is seen predominantly in patients with coexistent myxedema ascites (39), suggesting that right-sided heart failure may contribute to the liver dysfunction associated with hypothyroidism.

The clinical picture of hypothyroidism can be confused with that of advanced chronic liver disease because of similarities in presenting symptoms and laboratory findings (29,32). Myalgias, fatigue, muscle cramps, and elevated AST and lactate dehydrogenase (LDH) levels (resulting from myopathy in patients with hypothyroidism) are common to both liver disease and hypothyroidism. In addition, myxedema coma and ascites with a high serum–ascites albumin gradient can be seen in patients with hypothyroidism, in whom ascites may be the result of right-sided heart failure or increased permeability of the peritoneal membrane to proteins and mucopolysaccharides. Differentiating hypothyroidism from advanced chronic liver disease is crucial because hepatic dysfunction associated with hypothyroidism typically resolves within weeks of the initiation of thyroid replacement therapy (29,31,32).

Cushing's Syndrome

Excess serum cortisol results in the typical features of Cushing's syndrome: Weight gain, moon facies,

diabetes, and acne. Because obesity is a major clinical feature of this disorder, affected persons may develop mild to severe steatosis or steatohepatitis (29,40). Increased hepatic glycogen deposition and abnormalities in bile flow also have been described in patients with Cushing's syndrome.

Addison's Disease

Destruction of the adrenal glands, resulting in hypocortisolism, may lead to nonspecific elevations in serum levels of AST and LDH. These abnormalities typically return to normal after cortisol replacement. Centrilobular fibrosis has been described in liver biopsy specimens from patients with Addison's disease (29); however, the pathogenesis of this finding remains unclear.

Acromegaly

Organomegaly is characteristic of patients with acromegaly, who typically have hepatomegaly. Liver chemistry levels and liver function are typically within normal limits (29,41).

Celiac Disease

Although the manifestations of celiac disease (also known as *celiac sprue* or *gluten-sensitive enteropathy*) were once thought to be confined to the gastrointestinal tract, important extraintestinal manifestations of celiac disease have now been recognized. Liver chemistry abnormalities, typically characterized by mild elevations in serum levels of AST and ALT, have been described in up to 55% of patients with celiac disease, and elevations in the level of serum alkaline phosphatase have been described in approximately 5% of cases (42). Conversely, celiac disease has been diagnosed in as many as 9% of patients with otherwise unexplained liver chemistry abnormalities (43,44,45), and it has been suggested that testing for celiac disease be included in the evaluation of otherwise unexplained liver chemistry abnormalities (42). Liver biopsy specimens from patients with celiac disease and abnormal liver chemistries typically reveal nonspecific findings. In most cases, the liver chemistry abnormalities resolve after the institution of a gluten-free diet (42,43,46).

The mechanism leading to liver chemistry abnormalities in patients with celiac disease is uncertain. One theory suggests that increased permeability of the small intestine leads to the delivery of toxins to the liver through the portal circulation. Another theory suggests that chronic small bowel inflammation is the cause (42).

In addition to nonspecific liver biochemical elevations, celiac disease has been associated with other liver diseases. Epidemiologic data suggest that the frequency of primary biliary cirrhosis (PBC) in patients with celiac disease is 3%, whereas the frequency of celiac disease in patients with PBC is 6% (47,48,49). Other studies have suggested a possible relationship between celiac disease and primary sclerosing cholangitis (PSC), as well as autoimmune hepatitis and nodular regenerative hyperplasia; these links are weaker than those found between celiac disease and PBC (50,51). In contrast to the nonspecific hepatitis associated with celiac disease, the disease courses of PBC, PSC, and autoimmune hepatitis do not appear to be altered by the initiation of a gluten-free diet (42), although liver dysfunction and ascites have been reported to reverse (52).

Rheumatic Diseases

The frequency and clinical significance of hepatic abnormalities in rheumatic (collagen vascular) disorders are poorly defined, in part because liver biopsy is usually not performed in patients with mild liver biochemical test result abnormalities. In addition, the medications used to treat collagen vascular disorders may cause hepatotoxicity, and distinguishing drug-induced liver disease from liver disease associated with

the underlying systemic rheumatic disease may be difficult (53). Table 11.2 summarizes the typical features of liver disease associated with the various collagen vascular disorders.

Systemic Lupus Erythematosus

Hepatic involvement in systemic lupus erythematosus (SLE) has been difficult to characterize (53,54,55). Initially, hepatic manifestations of SLE were thought to be rare. However, a retrospective observational study performed by Runyon et al. in 1980 (56) described hepatomegaly in up to 39% of 238 patients with SLE, with associated jaundice in 24%. Overall, 21% of these patients had abnormal liver biochemical test and liver biopsy results that could not be explained by other causes.

Hepatic involvement in SLE may be caused by the underlying disease, a coexisting disorder, or medications. Differentiating autoimmune hepatitis (formerly called *lupoid hepatitis*) from SLE-related hepatic manifestations is based in part on the detection of smooth muscle antibodies in the serum of patients with autoimmune hepatitis; these antibodies are rare in patients with SLE (57). This distinction is important because patients with autoimmune hepatitis typically have more serious, progressive liver disease as compared with patients who have hepatic involvement in SLE. Coexisting liver disease such as viral hepatitis and PBC can be seen in patients with SLE (53).

Table 11.2. Hepatic Manifestations of the Major Collagen Vascular Diseases

Disease	Clinical and biochemical features	Histology
Systemic lupus erythematosus	Hepatomegaly	Steatosis
	Jaundice	Chronic hepatitis
	Raised ALT level	
Rheumatoid arthritis	Raised alkaline phosphatase level	Kupffer cell hyperplasia
	Raised γ-glutamyl transpeptidase level	Steatosis
Polymyositis	Jaundice	Chronic hepatitis (rare)
	Raised alkaline phosphatase level	Primary biliary cirrhosis
Sjögren's syndrome	Raised aminotransferase and/or alkaline phosphatase levels	Primary biliary cirrhosis Chronic hepatitis
	Jaundice	Cryptogenic cirrhosis
Scleroderma	Hepatomegaly	Cirrhosis
	Prolonged prothrombin time	Primary biliary cirrhosis
	Jaundice	
	Raised aminotransferase and/or alkaline phosphatase levels	
Antiphospholipid antibody syndrome	Budd-Chiari syndrome	Nodular regenerative hyperplasia
	Hepatosplenomegaly	

	Jaundice	
	Raised ALT level	
Polyarteritis nodosa	Raised ALT and AST levels	Lymphocytic infiltration in the intima and media of hepatic arteries

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Hepatomegaly is the most consistent clinical finding in patients with hepatic manifestations of SLE (53,58,59). Jaundice and splenomegaly have also been described. Nonspecific liver biochemical abnormalities with mild elevations of serum AST, ALT, bilirubin, and alkaline phosphatase levels have been described.

The most common hepatic histologic finding in patients with SLE is steatosis, often not attributable to glucocorticoid use or other risk factors for steatosis (53,60,61). Additional histologic findings include granulomatous hepatitis, centrilobular necrosis, chronic hepatitis, and, in rare cases, cirrhosis. Other reported findings in patients with SLE include microabscesses from bacterial infection, hemosiderosis from blood transfusion, and drug toxicity.

Patients with SLE and other rheumatic diseases such as juvenile rheumatoid arthritis (RA) are especially susceptible to salicylate toxicity from prolonged high-dose salicylate therapy (62,63). Salicylate hepatotoxicity in patients with SLE is characterized by diffuse hepatocyte injury, disruption of the limiting plate, stellate cell activation, a plasma cell–predominant chronic portal infiltrate with occasional lymphoid follicles, and fibrosis. A more acute pattern of salicylate injury is characterized by ballooning of centrilobular hepatocytes, scattered acidophilic bodies, and moderate portal inflammation.

Successful treatment of SLE or discontinuation of offending medications typically leads to improvement in associated hepatic biochemical and histologic abnormalities (53). Criteria for treating SLE-related liver disease are not well defined. Liver biochemical abnormalities improve in most patients treated with glucocorticoids. In patients who have persistent abnormalities in liver test results despite treatment, a thorough evaluation, including liver biopsy, should be considered. Attention should be paid to the patient's medication history, particularly salicylate use.

Rheumatoid Arthritis

Abnormalities in liver chemistries, primarily elevated serum alkaline phosphatase and γ-glutamyl transpeptidase levels, have been reported in up to 6% of patients with RA (53,55,64,65,66). Elevated alkaline phosphatase levels may also be of bone origin. Liver biopsy findings from patients with abnormal liver chemistries and RA are nonspecific and include Kupffer cell hyperplasia, steatosis, and a periportal mononuclear cell infiltrate (67,68). As in patients with SLE, medications used in the treatment of RA, such as methotrexate, gold salts, nonsteroidal anti-inflammatory drugs, glucocorticoids, methotrexate, and other disease-modifying antirheumatic drugs, may result in hepatotoxicity that is difficult to distinguish from hepatic disease caused by RA.

Relapsing Polychondritis

Relapsing polychondritis (RP) is an uncommon, multisystem disorder that is characterized by recurring inflammation of cartilaginous tissues, including the nasal, auricular, and laryngotracheobronchial cartilage. Associated autoimmune disorders have been described in up to 35% of affected patients. Comorbid liver diseases, including PBC and HCV infection, have been

described in patients with RP; however, these associations are infrequent, at the level of case reports (69).

Myositis

Polymyositis is an inflammatory myopathy, which when associated with a characteristic rash is known as *dermatomyositis*. These conditions can occur as isolated disorders but are associated with other rheumatic diseases in up to 30% of affected persons (53). Both dermatomyositis and polymyositis can occur as a paraneoplastic syndrome in approximately 10% of cases. Because these disorders cause autoimmune destruction of muscle tissue, elevated levels of serum ALT, AST, and LDH can often be seen. These abnormalities are usually associated with elevated levels of serum creatinine kinase, and because patients may also have nonspecific constitutional symptoms at presentation, primary liver disease may be suspected. In fact, liver involvement in patients with inflammatory myositis is rare. A possible association between myositis and PBC has been suggested (64), but case reports describing this association are confounded by the presence of other rheumatic disorders thought to involve the liver. Patients with polymyositis or

dermatomyositis and elevated levels of alkaline phosphatase should undergo serologic testing for PBC with antimitochondrial antibodies (53).

Sjögren's Syndrome

Among 300 patients with primary Sjögren's syndrome (keratoconjunctivitis sicca) investigated for liver involvement by Skopouli et al. 7% had abnormal liver biochemical test results (70). Of these patients, 6.6% had antimitochondrial antibodies, 92% of whom had features of PBC. Although the exact frequency of PBC in patients with primary Sjögren's syndrome is unknown, an overlap between the two conditions has been well described, perhaps related to the similar underlying pathogenic mechanisms of the two diseases (65,71) (see Chapter 24). Patients with primary Sjögren's syndrome and abnormal liver chemistries should undergo testing for antimitochondrial antibodies (53). HCV infection can be associated with lymphocytic sialadenitis, which superficially resembles Sjögren's syndrome.

Scleroderma

Although hepatic involvement in scleroderma is rare, limited cutaneous scleroderma, or the CREST (calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) syndrome, has been described in 3% to 17% of patients with PBC (72,73,74). The prevalence of PBC in patients with scleroderma is unknown. Scleroderma typically precedes PBC by a mean of 12 years (onset at age 36 years vs. 48 years). Raynaud's phenomenon is the most common presenting symptom. Anticentromere antibodies are relatively specific for the CREST syndrome and have been found in the serum of 10% to 29% of patients with PBC but in up to 100% of patients with both PBC and CREST syndrome. Up to 90% of patients with scleroderma and PBC also have evidence of Sjögren's syndrome; the acronym "PACK" (PBC, anticentromere antibodies, CREST, and keratoconjunctivitis sicca) has been used to describe this constellation (74).

Primary Antiphospholipid Antibody Syndrome

Primary antiphospholipid antibody syndrome (APS) is defined by the clinical features of recurrent arterial or venous thrombosis, recurrent pregnancy loss, and thrombocytopenia in the presence of antiphospholipid antibodies (anticardiolipin antibodies and the lupus anticoagulant). The diagnosis of APS requires the detection of antibodies on two separate occasions 6 months apart. The hepatic manifestations of APS are usually related to vascular abnormalities (53). Several cases of hepatic vein thrombosis (Budd-Chiari syndrome) have been reported in patients with APS (75,76). In addition, one report suggests a role for antiphospholipid antibodies in the pathogenesis of nodular regenerative hyperplasia of the liver (77), which is characterized by diffuse micronodular transformation of hepatic parenchyma resulting in elevated liver chemistries and portal hypertension (see Chapter 40).

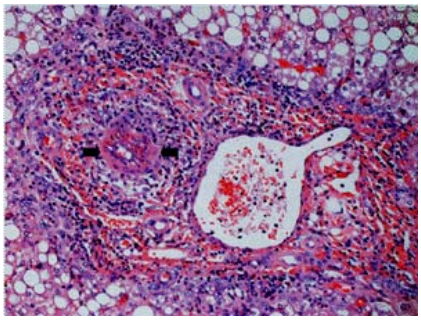
Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is a systemic vasculitis that primarily affects small- and medium-sized arteries. PAN is found in association with chronic HBV infection in over 50% of cases (78) (see Chapter 29). Nevertheless, PAN is a rare complication of HBV infection and occurs in only 1% to 5% of infected persons. Laboratory abnormalities in patients with PAN typically consist of mild elevations in serum levels of AST and ALT; cholestasis is a rare finding (79). Percutaneous liver biopsy specimens in patients with PAN rarely reveal the characteristic lymphocytic infiltration in the intima and media of the hepatic arteries (78), but this finding can be observed on larger specimens obtained at autopsy in up to 50% of patients (Fig. 11.3). Progressive liver disease in patients with PAN is usually not caused by PAN per se, but rather by chronic HBV infection (80,81). Treatment with an antiviral agent and plasma exchange has been recommended (82).

Adult Still's Disease

Rarely, adult patients may present with a syndrome that clinically resembles juvenile RA. Common presenting

symptoms of adult Still's disease include intermittent fever, pleuritis, seronegative polyarthritis, lymphadenopathy, splenomegaly, and pericarditis. Liver involvement in Still's disease is common; hepatomegaly may be identified in up to one half of affected persons (55). Elevated serum aminotransferase levels, up to five times normal, may be seen in up to 92% of patients, and increased serum alkaline phosphatase and bilirubin levels have been described in up to 65% of patients. Although liver biochemical abnormalities associated with Still's disease typically resolve with the initiation of treatment with salicylates, glucocorticoids, or other immunomodulators, fulminant hepatitis requiring liver transplantation has been described in rare cases (83).



▪ **Figure 11.3** Liver biopsy specimen from a patient with hepatic involvement in polyarteritis nodosa revealing a lymphocytic infiltrate in the intima and media of the hepatic artery (*arrows*) (hematoxylin and eosin). (Courtesy of John Hart, MD, Chicago, IL: Department of Pathology, University of Chicago Pritzker School of Medicine.)

Psoriasis

Several studies have reported an increased risk for the development of cirrhosis among persons with psoriasis (84,85). Although patients with psoriasis are often treated with hepatotoxic medications such as methotrexate (see Chapter 33), liver disease has been found to develop in patients with this disorder in the absence of medications, alcohol use, or viral hepatitis (86). The development of cirrhosis in patients with psoriasis has been attributed to the associated NASH (86). Several studies have suggested that insulin resistance may play a role in the pathogenesis of psoriasis (87) and, therefore, may be a pathogenic factor common to psoriasis and associated liver disease.

Sickle Cell Disease

Homozygous sickle cell anemia, or sickle cell disease (SCD), is estimated to affect 1 in 600 African American children. Liver biochemical abnormalities are universal in patients with SCD; most patients have elevated serum levels of unconjugated bilirubin secondary to ongoing hemolysis and elevated AST levels. Elevated serum alkaline phosphatase levels are rarely of hepatic origin (88).

Patients with SCD may commonly present with one of several acute hepatic syndromes that manifest clinically as fever, right upper quadrant pain, and jaundice (Table 11.3). The differential diagnosis includes *acute sickle hepatic crisis* (ASHC), *sickle cell intrahepatic cholestasis* (SCIC), cholecystitis, choledocholithiasis, and acute viral hepatitis (88). Patients with ASHC typically present with elevated serum levels of AST and ALT that are usually no higher than 300 U/L and serum bilirubin levels no higher than 15 mg/dL. The syndrome, caused by hypoxic injury to the hepatocytes from sinusoidal sickling, is self-limited and usually resolves within 14 days of providing therapy with intravenous fluids and analgesics. Liver biopsy is typically not required to make the diagnosis of ASHC (89,90,91).

In contrast to ASHC, SCIC is a rare complication of SCD that carries an ominous prognosis (88,92). The presenting clinical symptoms and signs are similar to those of ASHC. In addition, acute renal failure is usually found in patients with SCIC, and progressive encephalopathy, coagulopathy, and death are common.

SCIC may present as fulminant hepatic failure. ASHC is differentiated from SCIC on the basis of clinical and laboratory findings. In patients with SCIC, serum AST and ALT levels are usually greater than 1,000 U/L, and serum bilirubin levels also are strikingly high because of a combination of hemolysis, renal failure, and intrahepatic cholestasis. The underlying pathophysiology of SCIC is also related to sickling of red blood cells within the hepatic sinusoids and resulting anoxic hepatocyte damage. Liver biopsy specimens typically show ballooning of hepatocytes, sickled red blood cells in hepatic sinusoids, and intracanalicular cholestasis (Fig. 11.4). Treatment is largely supportive with the use of exchange transfusion (88,92,93,94,95), although successful liver transplantation for SCIC has been reported (96).

Table 11.3. Acute Hepatic Syndromes Associated with Sickle Cell Disease

Syndrome	Clinical findings	Laboratory findings	Treatment	Outcome
Acute sickle hepatic crisis	RUQ pain Nausea	Elevated AST and ALT levels	IV fluids Analgesia	Self-limited; complete

	Fever Jaundice Tender-hepatomegaly	(<300 U/L) Elevated bilirubin level (<15 mg/dL)		resolution in most patients
Sickle cell intrahepatic cholestasis	RUQ pain Nausea Vomiting Fever Jaundice	Markedly elevated AST and ALT levels Elevated alkaline phosphatase and lactate dehydrogenase levels Markedly elevated bilirubin level Prolonged prothrombin time Elevated creatinine level Leukocytosis	Exchange transfusion Liver transplantation	Usually fatal, although successful treatment with exchange transfusion and/or liver transplantation has been reported
Hepatic sequestration	RUQ pain Tender hepatomegaly	Falling hematocrit value	Exchange transfusion	Full recovery in most patients
		Liver chemistries typically normal		
Symptomatic cholelithiasis	Fever RUQ pain Jaundice	Elevated AST and ALT levels Leukocytosis Elevated bilirubin level	IV antibiotics Cholecystectomy Endoscopic retrograde cholangiopancreatography or common bile duct exploration for choledocholithiasis	Full recovery in most patients Postoperative acute chest syndrome in up to 10%

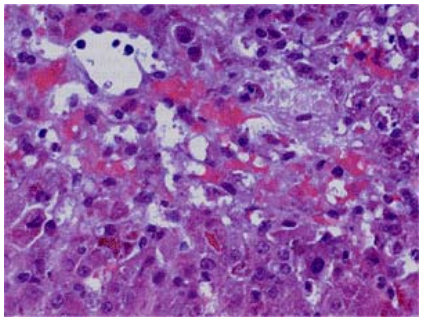
RUQ, right upper quadrant; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Patients with SCD are particularly prone to the development of pigmented gallstones, which develop in up to 58% of patients with SCD (88,97). Approximately 17% of patients are found to have choledocholithiasis at the time of cholecystectomy. In patients with SCD, acute cholecystitis or choledocholithiasis may be difficult to distinguish from ASHC. Because the prevalence of cholelithiasis in this patient population is very high, abdominal ultrasonography has limited specificity for diagnosing acute cholecystitis, although biliary scintigraphy may be helpful (98). Given the diagnostic difficulties, patients with SCD, gallstones, and symptoms suggestive of biliary pain or acute cholecystitis should be considered for cholecystectomy. However, cholecystectomy is not without risk in patients with SCD; the development of acute chest syndrome has been described in up to 10% of patients with SCD who undergo elective surgery (88,99).

Other acute liver diseases can be seen with increased frequency in patients with SCD. Sickling can lead to thrombosis within the hepatic veins and the Budd-Chiari syndrome (100). Although the frequency of hepatitis A is not reported to be increased in Western populations of patients with SCD, some reports have suggested that the course is more likely to be fulminant in patients with SCD than in those without SCD (101). Finally, hepatic sequestration crisis, in which sequestration of red blood cells within the liver leads to

massive hepatomegaly and a falling hematocrit value, has been described in patients with SCD. Although death from hepatic sequestration has been described, this syndrome is typically reversible with exchange

transfusion (88,102).



· Figure 11.4 Liver biopsy specimen obtained from a patient with severe sickle cell intrahepatic cholestasis. Note the presence of "ballooning" hepatocytes, cholestasis, and sickling of the red blood cells within the hepatic sinusoids (hematoxylin and eosin). (Courtesy of Gregory Lauwers, MD, Boston, MA: Department of Pathology, Massachusetts General Hospital.)

Chronic liver disease associated with SCD is also common. Autopsy series reveal cirrhosis in up to 29% of patients who die of complications from SCD (88,103). Most cases of chronic liver disease associated with SCD are thought to be secondary to hepatic iron overload after numerous blood transfusions or from infection by HBV or HCV acquired by the transfusion of contaminated blood products before the implementation of universal blood screening (88). Since the implementation of universal screening of donated blood in the United States, the incidence of viral hepatitis in patients with SCD has decreased substantially.

Amyloidosis

There are three major categories of amyloidosis, all of which may have hepatic manifestations. Primary or light-chain amyloidosis (AL) includes amyloidosis related to multiple myeloma. In secondary amyloidosis (AA), the amyloid fibrils form as a result of proteolytic cleavage of serum amyloid protein A, an acute-phase reactant. Causes of secondary amyloidosis include RA, osteomyelitis, tuberculosis, Hodgkin's disease and other lymphomas, familial Mediterranean fever, long-term heroin abuse, subclinical ankylosing spondylitis, and lepromatous leprosy. Familial amyloidosis is a rare disorder that includes type I familial amyloid polyneuropathy (FAP), an autosomal dominant disease caused by a mutation on chromosome 18 that leads to hepatic production of a variant prealbumin, transthyretin. The disease is manifested by a progressive mixed chronic polyneuropathy (sensory, motor, and autonomic) that presents after the age of 20 years and is invariably fatal. Early recognition is important because liver transplantation has been demonstrated to halt the progression of and even improve the neurologic symptoms (104).

Regardless of the type (AL, AA, FAP), the hepatic manifestations of amyloidosis are similar (105,106,107). Although hepatic amyloidosis is common and rarely causes clinical manifestations, it carries an ominous prognosis. The median survival in patients with biopsy-proven hepatic amyloidosis is 9 months, with 5- and 10-year survival rates of 13% and 1%, respectively. Clinical symptoms of hepatic amyloidosis are nonspecific and include weight loss, fatigue, abdominal pain, anorexia, early satiety, and nausea. Hepatomegaly is the most common physical finding in affected patients, and its presence is associated with a higher likelihood of abnormal liver chemistries.

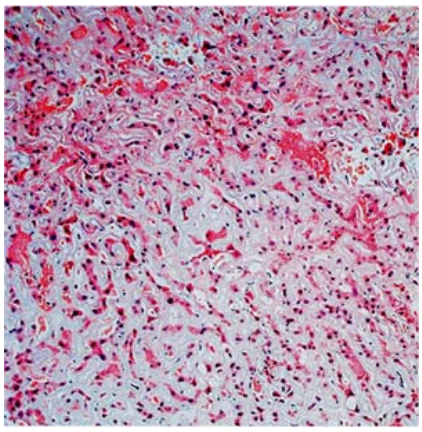
Abnormalities in liver chemistries are common in patients with hepatic amyloidosis. A series from the Mayo Clinic (106) described an elevated serum alkaline phosphatase level in 86% of patients with hepatic amyloidosis, with a level greater than 500 U/L in most cases. Levels of serum AST and bilirubin were found

to be elevated in 37% and 21% of cases, respectively, and the prothrombin time was prolonged in some cases.

The diagnosis of hepatic amyloidosis can be made by percutaneous liver biopsy, but this procedure has been reported to be associated with an increased bleeding risk in patients with hepatic amyloidosis (106,108). Amyloid appears as a pale pink homogenous amorphous protein on a liver biopsy specimen stained with hematoxylin and eosin (Fig. 11.5). Up to 95% of patients suspected of having hepatic amyloidosis have evidence of amyloid deposition on fat pad aspirate or bone marrow biopsy. Because the demonstration of hepatic involvement rarely changes the management, biopsy of lower-risk sites is usually warranted when the diagnosis of hepatic amyloidosis is in question.

Management of hepatic amyloidosis is limited primarily to supportive care or treatment of the underlying predisposing condition (106,108). A trial (109) comparing regimens containing melphalan, prednisone, and colchicine in patients with primary amyloidosis found that median survival was significantly longer in

patients treated with a combination of melphalan and prednisone than in those treated with colchicine alone or a regimen containing all three drugs. Liver transplantation may be of benefit in patients with FAP.



▪ **Figure 11.5** Liver biopsy specimen from a patient with hepatic amyloidosis. Notice the deposition of the amorphous pale pink protein throughout the parenchyma (hematoxylin and eosin). (Courtesy of John Hart, MD, Chicago, IL: Department of Pathology, University of Chicago Pritzker School of Medicine.)

Sarcoidosis

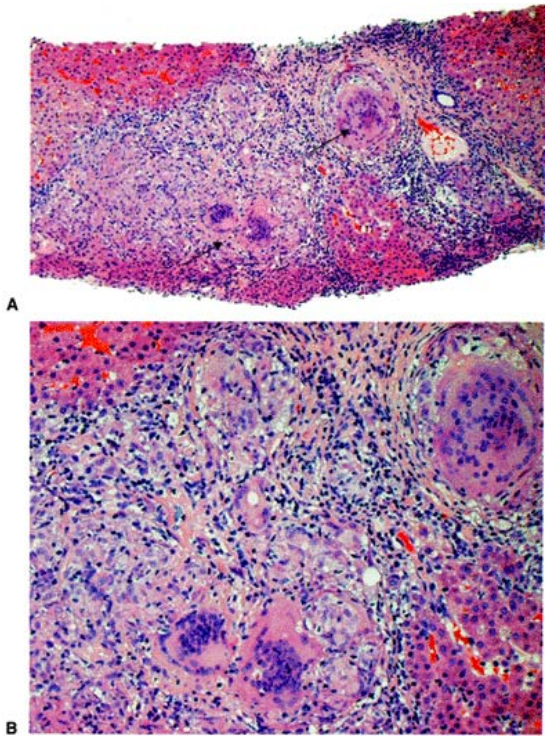
Sarcoidosis is a chronic systemic disorder of unknown etiology that is characterized by an accumulation of T lymphocytes and macrophages with granuloma formation in affected organs (see Chapter 52). Hepatic involvement is common in sarcoidosis and occurs in approximately 75% of all patients; however, liver dysfunction secondary to hepatic sarcoidosis is uncommon (110).

Physical findings are typically absent in patients with hepatic sarcoidosis. Evidence of mild hepatomegaly can be demonstrated by ultrasound or computed tomography in up to 50% of patients (110). The presence of splenomegaly and ascites does not always imply portal hypertension or hepatic involvement in patients with sarcoidosis. Splenomegaly is most commonly caused by granulomatous infiltration rather than portal hypertension. Ascites in patients with sarcoidosis can be related to cor pulmonale resulting from progressive pulmonary disease, hypoalbuminemia resulting from hepatic dysfunction, or, rarely, peritoneal sarcoid involvement.

Usually, serum immunoglobulin G levels are raised, the serum bilirubin level is normal, and the alkaline

phosphatase level is mildly elevated. Serum aminotransferase levels may or may not be elevated. The most common histopathologic finding in hepatic sarcoidosis is the presence of noncaseating granulomas, which typically predominate around portal tracts (111) (Fig. 11.6A,B).

In less than 1% of patients with hepatic sarcoidosis, progressive destruction of the intrahepatic bile ducts may lead to chronic intrahepatic cholestasis (112,113,114). Men are affected more commonly than women and may present with jaundice, pruritus, and constitutional symptoms such as fever and generalized malaise. Levels of serum alkaline phosphatase and γ-glutamyl transpeptidase are markedly elevated, and liver biopsy may reveal intracellular and intracanalicular cholestasis, portal inflammation, and fibrosis, in addition to noncaseating granulomas. Although an association may exist between sarcoidosis and PBC, antimitochondrial antibodies are not present in patients with sarcoidosis-related chronic intrahepatic cholestasis, and testing for antimitochondrial antibodies is recommended because of the similar clinical presentations of the two conditions. Other causes of cholestasis in patients with sarcoidosis include sarcoid involvement of the extrahepatic bile ducts, common bile duct compression by hilar lymphadenopathy, and pancreatic sarcoidosis (115,116).



▪ **Figure 11.6 A:** Low-power view of a liver biopsy specimen obtained from a patient with hepatic sarcoidosis. Noncaseating granulomas are seen (*arrows*). **B:** High-power view of the same specimen demonstrating multiple noncaseating granulomas (hematoxylin and eosin). (Courtesy of John Hart, MD, Chicago, IL: Department of Pathology, University of Chicago Pritzker School of Medicine.)

Cirrhosis and portal hypertension may develop in the later stages of hepatic sarcoidosis (110,117,118). Portal hypertension, often resulting from granulomatous phlebitis of intrahepatic veins, can develop in the absence of cirrhosis in up to 50% of patients (110,119). In approximately 25% of patients, portal hypertension is related to secondary biliary cirrhosis, although associated PBC or PSC may be present in rare cases. Patients with biliary cirrhosis secondary to hepatic sarcoidosis present with findings typical of chronic cholestasis, including hypercholesterolemia,

xanthomata, and osteomalacia. Treatment with glucocorticoids or low-dose methotrexate usually does not delay the progression of the liver disease but may improve constitutional symptoms and result in a decrease in the size of the liver if hepatomegaly is present (114,120). Treatment with ursodeoxycholic acid may improve cholestasis (121).

Fat-soluble vitamin deficiency, hypercholesterolemia, and the complications of portal hypertension require treatment. In patients with portal hypertension related to hepatic sarcoidosis, complications such as bleeding esophageal varices may be the initial clinical presentation. Management of esophageal varices associated with sarcoidosis includes nonselective β-blockers and endoscopic therapy (see Chapter 16). A transjugular intrahepatic or surgical portosystemic shunt may be considered in appropriate candidates (110). Patients with end-stage liver disease should be considered for liver transplantation (see Chapter 53).

Lymphoma

The liver is part of the reticuloendothelial system and may be involved by lymphomas. Although hepatic lymphomatous involvement is relatively common (122), life-threatening complications of lymphomas involving the liver are rare. A summary of the hepatic manifestations associated with lymphoma is shown in Table 11.4.

Hodgkin's Disease

Malignant hepatic infiltrates containing Reed-Sternberg cells have been described in up to 14% of patients with Hodgkin's disease diagnosed on clinical grounds and in up to 50% of patients diagnosed at autopsy; the frequency of hepatic involvement in Hodgkin's disease increases with disease progression (122,123,124). Patients with Hodgkin's disease may present with hepatomegaly, which occurs in approximately 9% of patients with stage I to II disease and in 45% with stage III to IV disease. Liver biochemical testing may reveal a mild increase in the serum ALT level and a moderate increase in the alkaline phosphatase level.

Neither physical findings nor abnormal liver chemistry results predict the presence of a Hodgkin's infiltrate in the liver. By definition, Hodgkin's disease involving the liver signifies stage IIIE or IV disease and has implications for prognosis and therapy. Therefore, patients in whom hepatic involvement is suspected should undergo liver biopsy (122,123,124).

Table 11.4. Hepatic Manifestations of Hodgkin's Disease and Non-Hodgkin's Lymphoma

	Hodgkin's disease	Non-Hodgkin's lymphoma
Clinical features	Hepatomegaly: Frequency increases with disease progression	Hepatomegaly: Common
	Jaundice: Usually related to intrahepatic biliary obstruction	Jaundice: Typically results from extrahepatic biliary obstruction at the level of the porta hepatis
	Acute liver failure: Rare	Acute liver failure: Rare, but more common than in Hodgkin's disease
Liver chemistries	Mild elevations of alanine aminotransferase and alkaline phosphatase levels are common	Aminotransferase levels may be elevated two to three times normal
	Elevated bilirubin levels can be seen	Mild elevations in alkaline phosphatase and bilirubin levels can be seen
Biopsy findings	Usually nonspecific: Lymphocytic infiltrate without histiocytes in the portal tracts; focal hepatocyte necrosis; Kupffer cell hyperplasia; slight iron deposition; moderate-to-severe steatosis; pleomorphic cell infiltrates; and noncaseating granulomas	Lymphomatous infiltrate; histology depends on the type of lymphoma
	Reed-Sternberg cells in rare cases	

Hepatic histologic abnormalities occur in up to 60% of patients with Hodgkin's disease and generally are nonspecific. In a series (123) of 308 patients with Hodgkin's disease who underwent 459 liver biopsies, Reed-Sternberg cells, which are diagnostic of Hodgkin's disease, were found in only 2.4% of liver biopsy specimens. Other findings included a lymphocytic infiltrate without histiocytes in the portal tracts (56%), focal necrosis (24%, but present on a repeat biopsy specimen in only 35% of the original group), Kupffer cell hyperplasia (52%), slight iron deposition (23%), moderate-to-severe steatosis (20%), pleomorphic cell infiltrates (4%), and noncaseating granulomas (2%). Thirty percent of patients had a normal biopsy result.

Jaundice, when present, is typically the result of intrahepatic biliary obstruction (125), although cholestasis in the absence of extrahepatic obstruction or significant tumor infiltration has been described. The cause of jaundice in such cases is unclear but may represent a paraneoplastic phenomenon, defect in hepatic microsomal function, or the vanishing bile duct

syndrome (126) (see Chapter 9). In rare cases, patients may present with jaundice and evidence of hepatic Hodgkin's disease on a liver biopsy specimen and no evidence of disease in peripheral lymph nodes (127). Such cases tend to be seen with the mixed cellularity and lymphocyte-depleted subtypes of Hodgkin's disease and have a poor prognosis. Jaundice has been shown to resolve in some patients after successful treatment of the underlying Hodgkin's disease, but persistent jaundice despite remission of the primary disease in other patients implies that permanent hepatic injury may have occurred (127).

Non-hodgkin's Lymphoma

Lymphomatous infiltration of the liver is more common in non-Hodgkin's lymphoma (NHL) than in Hodgkin's disease (122). Hepatic involvement with NHL has been described in liver biopsy specimens in up to 53% of cases (and an even higher percentage at autopsy), is more common with small-cell varieties than with large-

cell types, and has implications for staging, treatment, and prognosis (128). Liver biochemical test results may be abnormal (primarily an increase in serum alkaline phosphatase), and hepatomegaly may be detected. Rarely, NHL can present as a primary hepatic lymphoma (129). Apart from human immunodeficiency virus–associated lymphomas, primary hepatic lymphoma has a better prognosis than NHL because of the possibility of cure with successful resection. The yield of laparotomy for detecting hepatic involvement in NHL is higher than that for laparoscopy and for percutaneous needle biopsy of the liver, although the yield of needle biopsy may be increased with the use of ultrasound guidance (128).

Jaundice is rare in patients with NHL and in the past was considered a terminal occurrence (125). Jaundice secondary to extrahepatic biliary obstruction is more common in NHL (1.2%) than in Hodgkin's disease (0.3%), and biliary obstruction occurs most commonly at the porta hepatis, although primary lymphomatous involvement of the bile ducts has been reported in rare cases. In patients with NHL and jaundice, gallstones and pancreatic adenocarcinoma must be excluded. Evaluation may include computed tomography, magnetic resonance cholangiopancreatography, endoscopic retrograde cholangiopancreatography, endoscopic ultrasound, and laparotomy.

Acute Liver Failure Secondary to Lymphoma

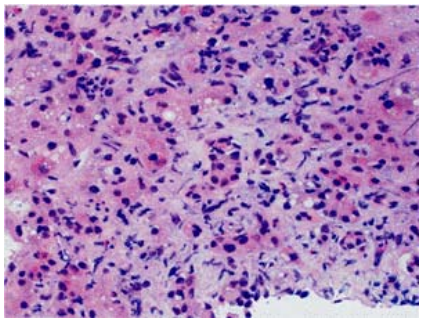
Rarely, malignant infiltration of the liver with lymphoma can cause acute liver failure (ALF) (122,127,130) (see Chapter 21). Malignant infiltration accounted for 0.44% of all cases of ALF seen at King's College Hospital from 1978 to 1995 (130). NHL accounted for 50% and Hodgkin's disease for 17% of the malignant causes of ALF in this series. Most patients in the King's College Hospital series presented with "hyperacute" liver failure (encephalopathy within 7 days of jaundice in the absence of previous liver disease), whereas the remainder evolved more slowly but within 28 days. Prodromal symptoms usually occurred 2 to 4 weeks (range, 5 days to 2 months) before the onset of liver failure; the most common prodromal symptoms were malaise (50%), weight loss (39%), right upper quadrant pain (39%), and fever (33%). Palpable lymphadenopathy was detected in five of nine patients with NHL; all patients had a firm palpable liver two to five fingerbreadths below the right costal margin, a rare finding with other causes of ALF. Laboratory tests could not distinguish these patients from those with ALF from other causes, and most patients had a rapid clinical deterioration, with death from multiorgan failure occurring in 94% of patients at a median of 6 days (range, 1 to 54 days) from admission. Histologic evaluation by needle biopsy of the liver or at autopsy demonstrated that the malignant infiltration was spread diffusely through the liver parenchyma, with large areas of necrosis in both infiltrated and noninfiltrated areas.

The pathogenesis of ALF caused by lymphomas and other malignancies includes massive hepatic replacement by nonfunctioning malignant cells, release of cytokines by tumor cells, Kupffer cell activation, and ischemia from engorgement of hepatic sinusoids with leukocytes and platelets. Prompt diagnosis with liver biopsy and treatment of the underlying malignancy during the prodromal period are the keys to a chance for a successful outcome. Differentiation of these patients from those with nonmalignant causes of ALF is essential because liver transplantation may be life saving in the latter but is contraindicated in the former.

Myelofibrosis with Myeloid Metaplasia

Myelofibrosis with myeloid metaplasia is a chronic myeloproliferative disorder that results from a clonal stem cell defect and leads to ineffective erythropoiesis, myelofibrosis, and extramedullary hematopoiesis. Portal hypertension with associated esophageal varices and ascites has been described in up to 7% of patients with this disorder (131). The mechanism appears to be increased portal flow resulting from the massive splenomegaly that can accompany myelofibrosis, in addition to thrombotic obstruction of the small intrahepatic portal veins (132,133). Patients with complications of portal hypertension can usually be managed successfully with splenectomy, although endoscopic variceal band ligation has been reported to be useful

in a small number of patients with myeloid metaplasia and bleeding esophageal varices (134,135,136).



▪ **Figure 11.7** Liver biopsy specimen demonstrating an inconspicuous infiltrate in a patient with

systemic mastocytosis. Immunostaining subsequently revealed the infiltrate to be composed primarily of mast cells (hematoxylin and eosin). (Courtesy of John Hart, MD, Chicago, IL: Department of Pathology, University of Chicago Pritzker School of Medicine.)

Stauffer's Syndrome

Stauffer's syndrome refers to paraneoplastic intrahepatic cholestasis occurring in the setting of a renal cell carcinoma (137,138,139,140). By definition, patients with Stauffer's syndrome have no evidence of liver metastasis or other causes of biliary obstruction. A paraneoplastic increase in serum alkaline phosphatase levels (with or without jaundice) has been described in up to 21% of patients with renal cell carcinoma and is thought to occur as a result of the production of cytokines, such as interleukin-6 and granulocyte-macrophage colony-stimulating factor, by the tumor (141). Elevations in serum bilirubin and alkaline phosphatase levels may normalize after nephrectomy, but the diagnosis of Stauffer's syndrome portends a poor overall prognosis.

Systemic Mastocytosis

Systemic mastocytosis is characterized by mast cell hyperplasia. The disease can involve multiple organ systems; the liver is involved in up to 61% of cases (142). Hepatosplenomegaly can be seen in up to 41% of patients with systemic mastocytosis involving the liver, and liver biochemical abnormalities, including elevated serum levels of aminotransferases, alkaline phosphatase, γ-glutamyl transpeptidase, and 5′-nucleotidase, have been described in up to 54%. Elevated serum alkaline phosphatase levels and hepatosplenomegaly have been found to correlate with the presence of mast cell infiltration on a liver biopsy specimen (Fig. 11.7). Portal fibrosis can be seen on liver biopsy specimens from affected persons, but cirrhosis has not been described, although portal hypertension has been reported, presumably from various combinations of increased portal venous flow (secondary to splenomegaly) (142), nodular regenerative hyperplasia, mast cell portal venous infiltration, and sinusoidal obstruction syndrome (119).

Annotated References

Abdo A, Meddings J, Swain M. Liver abnormalities in celiac disease. *Clin Gastroenterol Hepatol* 2004;2:107–112.

This paper provides a detailed review of the hepatic abnormalities found in patients with celiac disease. It also highlights the importance of testing for gluten enteropathy in patients with otherwise unexplained liver chemistry abnormalities and provides ample evidence to support this practice.

Abraham S, Begum S, Isenberg D. Hepatic manifestations of autoimmune rheumatic diseases. *Ann Rheum Dis* 2004;63:123–129.

This paper reviews the broad spectrum of liver diseases that can be associated with the various rheumatic diseases and the drugs used to treat them. The authors point out that, although hepatic manifestations of autoimmune disease are rare, the presence of liver chemistry abnormalities should prompt a thorough workup for potentially treatable coexistent liver disease.

Banerjee S, Owen C, Chopra S. Sickle cell hepatopathy. *Hepatology* 2001;33:1021–1028.

This article provides a concise and clinically helpful overview of both acute and chronic liver diseases that are seen in patients with sickle cell disease, including acute sickle hepatic crisis and sickle cell intrahepatic cholestasis. Many of the acute hepatic syndromes that are associated with sickle cell disease can have similar clinical appearances, and this paper describes the features that differentiate these disorders.

Blich M, Edoute Y. Clinical manifestations of sarcoid liver disease. *J Gastroenterol Hepatol* 2004;19:732–737.

This article describes the hepatic manifestations commonly found in patients with sarcoidosis. As a systemic granulomatous disease, sarcoidosis often affects the liver, although hepatic involvement is rarely symptomatic. The authors provide a detailed mechanistic explanation for the development of portal hypertension, cirrhosis, and cholestasis as a result of granulomatous involvement of the liver.

Tolman KG, Fonseca V, Tan MH, Dalpiaz A. Narrative review: hepatobiliary disease in type 2 diabetes mellitus. *Ann Intern Med* 2004;141:946–956.

This paper critically reviews the association between type 2 diabetes mellitus and liver disease. The potential role of insulin resistance in the pathogenesis of diabetes-associated liver disease is explored in detail, and

the association between hepatitis C and diabetes mellitus is discussed.

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Chapter 12

Hematologic Disorders and the Liver

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Key Concepts

- In liver disease, red blood cells (RBCs) acquire membranes that are rich in cholesterol, with diminished membrane fluidity and impaired ability to repair oxidatively damaged RBC membrane phospholipids that arise during normal RBC senescence. As a consequence, altered peripheral blood RBC morphologies occur (target cells, echinocytes, acanthocytes, spur cells, and stomatocytes) and RBC survival is shortened. This form of "extrinsic nonimmune hemolytic anemia" is accentuated by hypersplenism when splenomegaly due to portal venous hypertension ensues from severe hepatocellular dysfunction.
- Patients with primary RBC abnormalities that are either inherited (e.g., thalassemia, sickle hemoglobinopathies) or acquired (e.g., myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria) can develop liver disease. Frequently, this relates to management of the hematologic disorder, such as RBC transfusions resulting in viral hepatitis or iron overload. However, the primary hematologic disorder itself can cause intrahepatic disease, as in sickle cell disease in which intrahepatic sickling causes ischemia with development of focal necrosis, portal fibrosis, and cirrhosis or hepatic sequestration crisis. Chronic hemolysis also produces hepatobiliary complications from pigmented gallstones.
- Myeloproliferative disorders (MPDs), including polycythemia vera, essential thrombocythemia, chronic myelogenous leukemia, and agnogenic myeloid metaplasia (AMM), are all associated with extramedullary hematopoiesis. EMH may occur in any organ, including liver and spleen. With increasing liver EMH, palpable hepatomegaly develops, with portal vein hypertension arising from increased splanchnic flow due to splenomegaly and/or EMH-associated intrahepatic obstruction. Portal vein thrombosis and Budd-Chiari syndrome from thrombotic occlusion or partial occlusion of hepatic veins and the inferior vena cava are complications of MPD; these should suggest the possibility of latent MPD if it is not already evident. PNH, another acquired hematopoietic disorder, can cause recurring hepatic vein thrombosis and Budd-Chiari syndrome, although the mechanism of thrombotic diathesis in PNH is unknown.
- Thrombocytopenia is common in chronic liver disease. Thrombocytopenia can relate to "distributional" factors, with platelet sequestration in a spleen that is enlarged because of portal vein hypertension. However, reduced level of thrombopoietin, which is produced constitutively by the liver, is the chief cause of thrombocytopenia from chronic liver disease. Immune or nonimmune processes causing underproduction thrombocytopenia and/or platelet destruction may accompany acute and chronic liver diseases. In HELLP (hemolysis with a microangiopathic blood smear, elevated liver enzyme levels, and low platelet concentration in pregnancy) syndrome, complications include hepatic failure,

hepatic infarction, and hepatic hematoma with or without rupture.

- The basis of coagulopathy of liver disease is often multifactorial including thrombocytopenia, factor deficiency (both antigenic and functional), and accelerated fibrinolysis. Despite the progressive nature of coagulopathy associated with advancing liver disease, the degree of coagulopathy has not been shown to be predictive of bleeding outcomes, and therefore, administration of plasma products is most appropriately reserved for instances of active bleeding or invasive procedures.
- In placebo-controlled trials, antifibrinolytic agents have been shown to decrease bleeding complications associated with orthotopic liver transplantation.

The pathologic processes affecting the liver often have profound influences on hemostatic and hematopoeitic systems. This intricate relationship is in part related to the vital function that the liver plays in synthesizing components of the coagulation pathways but also reflects the wide range of influence the liver has on hematopoeitic cells such as red blood cells (RBCs) and platelets. Conversely, patients suffering from many primary hematologic disorders such as sickle cell disease (SCD), myeloproliferative diseases (MPDs), and paroxysmal nocturnal hemoglobinuria (PNH) often suffer hepatic complications. This chapter describes the reciprocal nature of the disease affecting these two organ systems.

Red Blood Cell Abnormalities Caused by Liver Disease

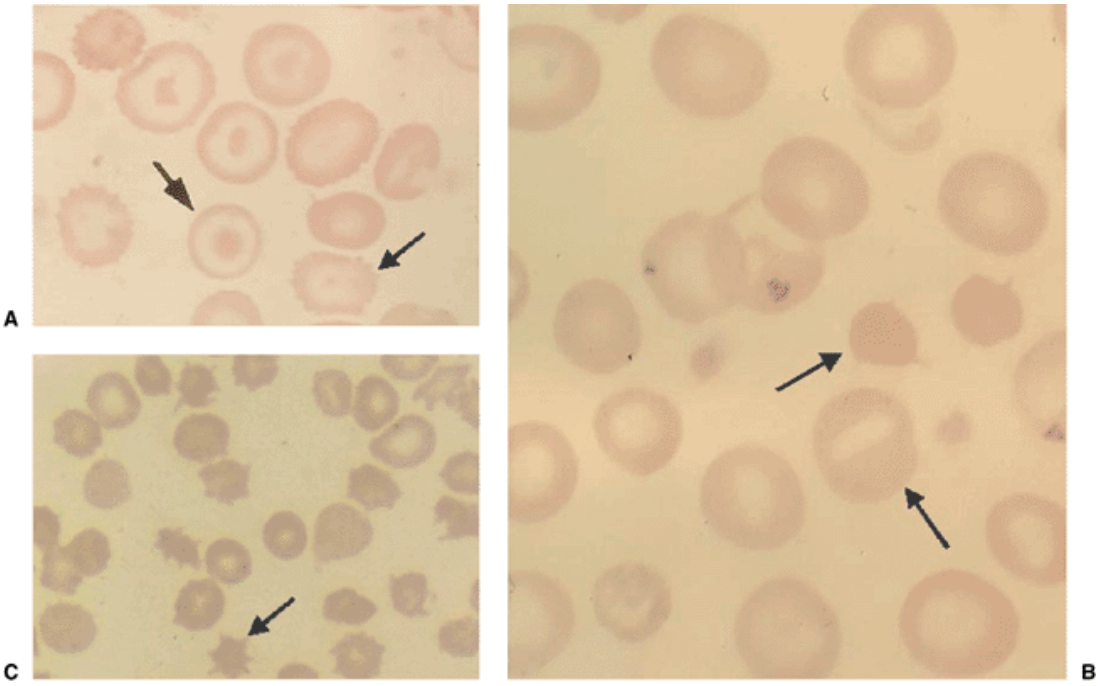
In liver disease, RBCs undergo membrane changes that shorten their survival. Shorter RBC survival defines hemolysis. Because liver disease, rather than immunologic processes, causes these RBC membrane changes, anemia accompanying liver disorders represents a form of "extrinsic nonimmune hemolytic anemia." When severe hepatocellular dysfunction produces portal venous hypertension and splenomegaly, hypersplenism can increase RBC destruction (1,2).

Laboratory signs of hemolysis include increased concentrations of serum indirect bilirubin and lactate dehydrogenase and decreased concentration of serum haptoglobin. Hemolysis in the absence of liver disease generally yields total serum bilirubin concentrations no higher than 5 to 6 mg/dL, but total bilirubin levels in hemolysis superimposed on hepatocellular dysfunction may exceed 7 mg/dL. The chief site of RBC loss is extravascular, within the liver and spleen. Therefore, laboratory signs of intravascular hemolysis (e.g., increased concentrations of plasma free hemoglobin, hemoglobinuria, and hemosiderinuria) are absent.

RBC membrane changes occurring in liver disease yield altered peripheral blood RBC morphologies, including target cells, echinocytes (burr cells), acanthocytes, spur cells, and stomatocytes (Fig. 12.1). As in hyperlipidemia, target cells (Fig. 12.1A) in liver disease (particularly cholestatic liver disease) arise from increased lipid deposition on RBC membranes, resulting in expanded RBC membrane surface area in relation to RBC cytoplasm (1,2). In contrast to thalassemic target cells, which are microcytic with RBC mean cell volumes (MCVs) less than 80 fL, the target cells from liver disease are macrocytic, with MCVs ranging from 100 to 110 fL. Notably, alcoholism induces macrocytosis, independent of liver disease and folate and/or cobalamin deficiency (3). This relates to RBC membrane changes caused by aldehyde adducts from acetaldehyde, a metabolite of alcohol (4,5). Echinocytes (named after sea urchins, which they resemble) have small undulating crenations or serrations uniformly distributed over the entire RBC membrane surface (Fig. 12.1A). While echinocytes can appear as artifacts on blood smears, particularly when albumin concentrations are low, they are common in advanced renal failure and liver disease. In liver disease, the echinocyte morphology may arise from abnormal high-density lipoprotein interacting with receptors on the RBC

membrane surface (6). In contrast to echinocytes, *acanthocytes*

display only a few "thorny" spicules, projecting at irregular intervals from the RBC membrane surface (Fig. 12.1B). *Spur cells* are acanthocytes that have more spherocytic shapes and blunted spicules (Fig. 12.1C), caused by RBC membrane loss from splenic molding (2,7). Increased RBC membrane proteolytic activity, caused by a circulating factor present in spur cell plasma, may contribute to spur cell formation (8). Although hypersplenism accentuates spur cell formation, prompting the consideration of splenectomy in patients with *spur cell hemolytic anemia*, liver transplantation is the definitive intervention (9). *Stomatocytes*, when seen in large numbers on peripheral blood smear (and therefore not likely related to smear artifact), indicate acute ethanol intoxication or severe liver disease, particularly that caused by alcohol (10). With their "mouth-shaped" areas of central pallor (Fig. 12.1B), stomatocytes are RBCs with reduced surface area in relation to volume, en route to becoming spherocytes. Regardless of specific RBC morphology, abnormal RBC shapes and shorter survivals in the setting of liver disease appear to relate to RBC membranes rich in cholesterol, with diminished fluidity and impaired ability to repair oxidatively damaged RBC membrane phospholipids that arise during normal RBC senescence (1,11).



▪ **Figure 12.1** Peripheral blood red blood cell (RBC) morphologies in liver disease— **(A)** target cell (↘) and echinocyte (↙); **(B)** acanthocyte (↗) and stomatocyte (↖); and **(C)** spur cell (↙).

Liver Disease Caused by Red Blood Cell Abnormalities

Patients with primary RBC abnormalities that are either inherited (e.g., thalassemia, sickle hemoglobinopathies) or acquired (e.g., myelodysplastic syndromes, PNH) can develop liver disease. Frequently, this relates to management of the hematologic disorder, such as RBC transfusions resulting in viral hepatitis or iron overload. However, the primary hematologic disorder itself can cause intrahepatic disease, as in SCD. Chronic hemolysis causing pigmented gallstones can also produce hepatobiliary complications.

In SCD, vaso-occlusive complications from the sickling process yield hepatic abnormalities because of ischemia, independent of SCD management. "Sickle cell hepatopathy" (12) refers to hepatic dysfunction arising from the collective effects of

vaso-occlusion and disease management (e.g., viral hepatitis and iron overload). Patients with homozygous hemoglobin (Hb SS) are more likely to develop sickle cell hepatopathy than are patients with the compound heterozygous hemoglobinopathies Hb SC and Hb S β-thalassemia. Cirrhosis is a frequent consequence (13).

In SCD, it is predominantly the level of unconjugated bilirubin that is elevated, with levels less than 6 mg/dL, correlating with lactic dehydrogenase (LDH) levels caused by chronic hemolysis. Aspartate aminotransferase (AST) levels correlate with LDH levels as well, also caused by hemolysis. With sickle hepatic crisis, bilirubin levels may double, and a rise in alanine aminotransferase (ALT) levels reflects hepatic injury (12). Because vascular complications affecting bone can cause high alkaline phosphatase levels, serum 5'-nucleotidase levels are better indicators of hepatic injury, correlating with ALT and γ-glutamyltransferase levels (14). Hyperammonemia in patients with cirrhosis may be accentuated by zinc deficiency, which results from renal tubular losses of zinc in sickle cell nephropathy (15) and deferoxamine therapy of iron overload (16). Zinc is a cofactor of the urea cycle enzyme, ornithine transcarbamylase, and zinc supplementation may benefit patients with SCD who develop zinc deficiency (17,18). Hepatic dysfunction in SCD may contribute to low protein C and protein S levels, thereby increasing the risk of stroke (19,20).

Hepatic histologic findings in SCD include intrasinusoidal sickling with proximal sinusoidal dilatation, Kupffer cell hyperplasia with erythrophagocytosis, hemosiderosis, focal necrosis, portal fibrosis, regenerative nodules, and cirrhosis (13). Radiologic assessments generally show hepatomegaly (presumably because of hyperplasia of the reticuloendothelial system) and either a small, atrophic spleen, as in Hb SS, or an enlarged spleen with infarcts, as in Hb SC and Hb S β-thalassemia. Magnetic resonance imaging (MRI) shows decreased signal intensity in the liver and spleen (before atrophy) because of iron deposition.

Acute sickle hepatic crisis (12), arising from ischemia caused by sinusoidal obstruction, presents as right upper quadrant pain, nausea, low-grade fever, tender hepatomegaly, and worsened jaundice, with aminotransferase and bilirubin levels less than 300 IU/L and 15 g/dL, respectively. Supportive treatment with intravenous hydration and analgesia generally improves symptoms and liver biochemical abnormalities within several days to weeks. However, acute sickle hepatic crisis can evolve to intrahepatic cholestasis if sickling within hepatic sinusoids leads to ischemia, hypoxia, and intracanalicular cholestasis in damaged, swollen hepatocytes. Leukocytosis, markedly worsening hyperbilirubinemia, renal failure, coagulopathy, and encephalopathy suggest this complication. Aminotransferase and bilirubin levels (mostly conjugated) may be strikingly high, exceeding 1,000 IU/L and 15 g/dL, respectively. Exchange transfusions and management of the accompanying coagulopathy can save lives (21,22).

Hepatic sequestration crisis (12), due to sequestration of large numbers of RBCs in the liver (as well as spleen and lung vasculature), presents as right upper quadrant abdominal pain with a rapidly enlarging liver and declining hematocrit (Hct) levels. Liver biochemistries may not necessarily worsen. Management includes improving oxygenation (including hyperbaric oxygen), transfusing packed RBCs, and considering exchange transfusions, particularly if pulmonary complications ensue with progressive hypoxia. With resolution of hepatic sequestration crisis, hepatic size regresses and Hct levels rise, reflecting mobilization of "undigested" sequestered RBCs from liver to peripheral blood. If the Hct level rises substantially ("reverse sequestration"), hypervolemia, hypertension, heart failure, and intra cerebral hemorrhage may occur, resulting in death (23).

Although hydroxyurea therapy decreases the frequency of vaso-occlusive pain crises and acute chest syndrome, it may not affect the frequency of hepatic sequestration crises (24). For patients with end-stage liver disease from SCD who are candidates for liver transplantation, exchange transfusions can maintain Hb S levels below 25%, in an

effort to reduce vaso-occlusive sickling damage to the graft (25).

Myeloproliferative Disorders and the Liver

MPDs, including polycythemia vera (PV), essential thrombocythemia (ET), chronic myelogenous leukemia (CML), and agnogenic myeloid metaplasia ([AMM] alias idiopathic myelofibrosis), are all associated with extramedullary hematopoiesis (EMH). EMH may occur in any organ, including liver and spleen. Of the various MPDs, EMH is most pronounced in AMM and least notable in CML. Whereas untreated CML always transforms into acute leukemia, PV and ET can eventually transform into fibrotic states called *postpolycythemic myeloid metaplasia* (*PPM*) and *post-thrombocythemic myeloid metaplasia* (*PTM*), respectively. With increasing liver EMH, palpable hepatomegaly can develop, with portal vein hypertension arising from increased splanchnic flow caused by splenomegaly and/or intrahepatic obstruction associated with EMH. Complications include ascites, esophageal and gastric varices, gastrointestinal bleeding, hepatic encephalopathy, portal vein thrombosis, and Budd-Chiari syndrome from thrombotic occlusion or partial occlusion of hepatic veins and the inferior vena cava (26,27,28). When splenectomy is performed in AMM, hepatic EMH may increase substantially, presumably as compensation for the loss of EMH in the spleen. The consequences include increasing hepatomegaly, worsening liver biochemistries (chiefly, alkaline phosphatase, bilirubin, and γ-glutamyltranspeptidase), and acute

hepatic failure (29). In contrast, splenectomy in CML is generally well tolerated without hepatic complications.

Development of the Budd-Chiari syndrome should always raise suspicion of an underlying MPD (28) with or without an accompanying hypercoagulable state, including factor V Leiden, prothrombin gene mutation, antiphospholipid antibodies, antithrombin deficiency, protein C deficiency, and protein S deficiency (30). Latent MPD may be detected in individuals with "idiopathic" Budd-Chiari syndrome by assessing various attributes of the MPDs, including spontaneous erythroid colony formation in the presence of low serum erythropoietin levels, increased megakaryocyte colony growth, and clonal karyotypic abnormalities evident by X chromosome inactivation patterns (31). Alternatively, molecular techniques can identify the acquired single gain-of-function point mutation (Val617Phe, V617F) involving Janus kinase 2 (JAK2) present in 65% to 97% of patients with PV, 23% to 57% of those with ET, and 43% to 57% of those with AMM (32).

Paroxysmal Nocturnal Hemoglobinuria and Hepatic Vascular Complications

PNH is an acquired hematopoietic disorder characterized by a defect in the glycosylphosphatidylinositol (GPI) anchor due to an abnormality in the *phosphatidylinositol glycan A* (*PIG-A*) gene. As a consequence, hematopoietic cells derived from PNH cells partially or completely lack certain GPI-linked proteins, including regulators of complement activation, CD59 (also called *membrane inhibitor of reactive lysis, protectin*, and *membrane attack complex inhibitory factor*) and CD55 (decay accelerating factor). Venous thrombosis of any site, including hepatic veins, can complicate PNH, although it is not known whether dysregulated complement activation (versus other mechanisms) contributes to this thrombotic diathesis.

Acute thrombosis in PNH is managed in a manner similar to venous thrombosis in other settings (33). Recurring hepatic vein thrombosis ultimately causes cirrhosis, and development of the Budd-Chiari syndrome portends a poor prognosis (34). Thrombolysis in hepatic vein thrombosis can improve long-term outcomes (35).

Thrombocytopenia in Liver Disease

Thrombocytopenia is common in chronic liver disease, although thrombocytosis may

arise when liver disease occurs with one or more known causes of "reactive" increases in platelet production (e.g., acute bleeding, iron deficiency, bacterial infection, or malignancy), asplenia, or hyposplenia. Thrombocytopenia can relate to "distributional" factors, with platelet sequestration in a spleen that is enlarged because of portal vein hypertension. However, reduced levels of thrombopoietin, which is produced constitutively by the liver, is the chief cause of thrombocytopenia from chronic liver disease (36). Additional causes of underproduction that may worsen thrombocytopenia in chronic liver disease include suppression or damage of bone marrow by viral infections (e.g., varicella, parvovirus, Epstein Barr virus, or human immunodeficiency virus), drugs, or toxins (e.g., alcohol, chemotherapy, or radiation therapy); nutritional deficiencies (e.g., folate or cobalamin); and acquired disorders of hematopoiesis (e.g., bone marrow aplasia or hypoplasia, or myelodysplastic syndromes).

Immune or nonimmune processes may destroy platelets, as is the case in idiopathic immune thrombocytopenic purpura, drug-induced thrombocytopenia, alloimmune destruction of platelets (e.g., posttransfusion, post-transplantation), disseminated intravascular coagulation (DIC), antiphospholipid antibody syndrome, physical destruction of platelets (from cardiopulmonary bypass, giant cavernous hemangiomas), thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP-HUS), HELLP (hemolysis with a microangiopathic blood smear, elevated liver enzymes, and low platelets in pregnancy) syndrome, and infection-associated thrombocytopenia. In sepsis, thrombocytopenia results from immune-mediated platelet destruction, hemophagocytic histiocytosis, and DIC (37).

Transfusion for massive blood loss, as may happen in patients with bleeding varices from portal vein hypertension, can cause "dilutional" thrombocytopenia. In one study, 75% of patients transfused with 20 or more RBC units in 24 hours developed platelet counts less than 50,000/μL, whereas none of the patients receiving less than 20 RBC units developed platelet counts below this level (38). Dilutional thrombocytopenia is distinguishable from posttransfusion purpura (PTP), which is an uncommon immune-mediated transfusion reaction that occurs primarily in women sensitized to a foreign platelet antigen by pregnancy. Patients sensitized by prior platelet or platelet-containing RBC transfusions are also at risk. The most common antigen implicated in PTP is human platelet antigen 1a (HPA-1a), formerly named *PIA1*. In PTP, severe thrombocytopenia arises 5 to 10 days after transfusion of platelets or platelet-containing RBCs and lasts days to weeks (39).

When thrombocytopenia is detected, examining peripheral blood smears allows clinicians to dismiss spurious thrombocytopenia, arising from platelet clumping in vitro. This arises from either insufficient

anticoagulation of the collected blood sample (excess ethylenediaminetetraacetic acid [EDTA]) or EDTA-dependent agglutinins.

Hemolysis with Microangiopathic Blood Smear, Elevated Liver Enzymes, and Low Platelets in Pregnancy

About 15% of pregnant women with pre-eclampsia (i.e., hypertension, proteinuria, and edema in pregnancy) develop thrombocytopenia; up to one third of these women experience severe thrombocytopenia with platelet counts less than 50,000/μL (40). As defined in a large case series, HELLP syndrome is the presence of microangiopathic hemolysis, with platelet counts less than 150,000/μL, serum LDH greater than 600 IU/L, and serum AST greater than 40 IU/L (40). Most patients present with midepigastric or right upper abdominal pain and nausea and/or vomiting. A small number of patients are asymptomatic (40). Patients with pre-eclampsia tend to have more frequent and severe thrombocytopenia, and signs of DIC occur in nearly 20% of patients (40). While HELLP syndrome can complicate pre-eclampsia, 15% to 20% of

patients with HELLP do not have hypertension or proteinuria (40). Hence, HELLP syndrome and pre-eclampsia may have separate etiologies, as underscored by the finding that fetuses of some women affected by HELLP syndrome have long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD) (41). LCHAD contributes to the acute fatty liver of pregnancy (41). However, in contrast to HELLP syndrome, acute fatty liver of pregnancy is not typically associated with thrombocytopenia unless DIC occurs (42).

Hematologic manifestations of pre-eclampsia and HELLP syndrome usually develop in the third trimester of pregnancy. In most patients, delivering the fetus is effective treatment, with hematologic abnormalities usually resolving within 3 days. However, 30% of women with HELLP syndrome develop hematologic abnormalities postpartum, usually within 48 hours of delivery, although sometimes it takes as long as 7 days after delivery. In both antepartum and postpartum HELLP, parenteral corticosteroids (betamethasone or dexamethasone) achieve more rapid improvement in clinical and laboratory parameters (43). When given antepartum, corticosteroid therapy can help delay parturition. In some patients, however, clinical and laboratory manifestations of HELLP worsen or do not improve after 3 days. In such patients, HELLP syndrome is indistinguishable from TTP-HUS, and plasma exchange should be initiated promptly, particularly if neurologic symptoms or signs and/or renal failure appear (44).

Additional complications of HELLP syndrome include hepatic failure (also seen in acute fatty liver of pregnancy), hepatic infarction, and hepatic hematoma with or without rupture. Antithrombin III levels decline as thrombocytopenia worsens (45), and underlying procoagulant states (e.g., antiphospholipid antibodies and factor V Leiden) may influence the risk of hepatic failure and infarction (46,47,48), marked by substantial elevations in the levels of aminotransferases. As platelet counts fall below 20,000/µL, patients face significant risks of hepatic hematoma. Although platelet transfusion may be advisable, there is no evidence that prophylactic platelet transfusions prevent this complication. Computed tomography (CT) and MRI are more sensitive than ultrasonography in detecting hepatic hematoma (49). When hematomas are contained, treatment is supportive; with rupture, patients may require orthotopic liver transplantation (50).

Decreased Synthesis of Coagulation Factors

The liver is the site of synthesis of coagulation factors. Prolongation of clotting tests (i.e., prothrombin time [PT], partial thromboplastin time [PTT]) is apparent only when levels of plasma coagulation factors are severely depressed (<30% to 40% of normal). The sensitivity of the PT as a marker of liver dysfunction reflects the relative influence of coagulation factors with shorter half lives including factor VII (4 to 6 hours) and factor V (12 hours). With advancing disease there are additional decrements in other factors including factors II, IX, and X.

Expression of factor VII on hepatocytes appears to be depressed even in the early stages of liver disease (51), but a measurable decline in circulating plasma levels usually represents more advanced disease (52). This has led to the evaluation of circulating factor VII as a prognostic marker (53) and eventually to the adoption of PT prolongation as an indicator of severity of disease (54,55). More recently, the prognostic Model for End-Stage Liver Disease (MELD) includes the international normalized ratio (INR) as an independent variable (56). INR reflects the ratio of an individual's PT relative to the standardized PT of the thromboplastin reagent to reduce the interlaboratory variability of PT measurements. However, INR was developed to monitor the hemostatic effects of oral anticoagulants and not the degree of liver dysfunction. In fact, studies have shown that in liver failure INR failed to identify a standardized PT independent of the thromboplastin reagent used (57).

Factor VIII is synthesized by both endothelial cells and hepatocytes, which accounts for the lack

of correlation between plasma concentration and the degree of liver dysfunction. Indeed, factor VIII levels are often elevated in patients with advanced liver disease, although the biological basis for this increase is not clear (58). Factor VIII is an acute-phase reactant and its increased concentrations are commonly seen in inflammatory disorders or acute infection; however, in the case of liver disease the levels do not appear to correlate with similar changes in other inflammatory markers such as C-reactive protein (59). It has also been hypothesized that elevations in von Willebrand's factor or low-density lipoprotein receptor–related protein levels observed in liver disease can stabilize circulating factor VIII, resulting in increased concentrations (60). Alternatively, portal hypertension after long-standing liver disease results in an increased number of sinusoidal endothelial cells that synthesize factor VIII (60).

Functional Deficiencies of Coagulation Factors

The activity of multiple coagulation factors including factors IX, VII, X, and prothrombin is dependent on the vitamin K–mediated conversion of glutamic acid into γ-carboxyglutamic acid in the *N*-terminal region of the proteins. Vitamin K deficiency has been implicated as a factor contributing to the coagulopathy of liver disease because of malabsorption and antibiotics usage. Vitamin K is derived from both dietary sources (green vegetables) and intestinal microflora synthesis. The absorption of Vitamin K requires pancreatic enzymes for its liberation from protein complexes and bile salts to facilitate its incorporation into micelles. Predictably, vitamin K absorption is impaired in cases of severe pancreatic insufficiency or extrahepatic biliary obstruction (61,62), but, in cases of intrinsic liver disease, the contribution of vitamin K deficiency appears to be relatively minor.

In severe hepatic disease, there are circulating coagulation factors that are deficient in γ-carboxyglutamic acid, which are commonly due to altered hepatocyte function. In one study, over 90% of patients with liver disease had detectable levels of circulating undercarboxylated prothrombin irrespective of bleeding symptoms or prolongation of PT. Treatment with vitamin K did not correct the prothrombin activity, suggesting that vitamin K deficiency was not the underlying etiology (63). Abnormally carboxylated forms of prothrombin (des-carboxyprothrombin) have also been documented in patients with hepatocellular carcinoma, which may be due to altered gene expression of the γ-carboxylase enzyme in the malignant clones (64,65). Nevertheless, vitamin K is administered to about 25% of hospitalized patients with cirrhosis in an attempt to correct the underlying coagulopathy (66). Although such a practice is considered low risk, with an incidence of anaphylaxis of roughly 3 per 10,000 intravenous doses (67), the benefit of vitamin K has not been evaluated in any randomized controlled trial (68). In a few small studies there has been some laboratory improvement in either PT or the amount of des-carboxyprothrombin in patients treated with vitamin K (59,69).

Functional abnormalities affecting fibrinogen are also well described in patients with advanced liver disease. Clot formation is dependent on the conversion of fibrinogen to fibrin, which is affected by the number of sialic acid residues present on the fibrinogen molecule. In liver failure, there is an increased fibrinogen sialylation, which impairs the ability of fibrin monomers to polymerize (70,71,72).

Consumptive Coagulopathy

The hemostatic abnormalities apparent in advanced liver disease extend beyond the synthesis of functional procoagulants in the hepatocyte. The deposition of fibrin polymers, which represents the final step in the coagulation pathway, depends on the delicate balance of coagulation factor activation, inhibition, and disintegration. In advanced liver disease, perturbations in each of these hemostatic components have been demonstrated.

Laboratory abnormalities that suggest a consumptive coagulopathy, otherwise referred

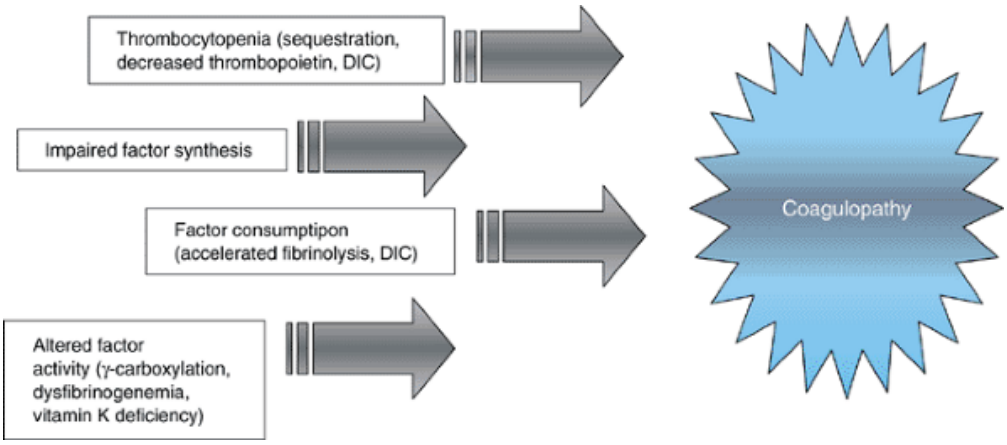
to as *DIC*, can be observed in patients with advanced liver disease (73,74). However, there is an on-going debate as to whether DIC is the consequence of advanced liver disease, a laboratory artifact, or the result of a heightened susceptibility to a secondary insult such as infection (74,75,76,77). Supporting evidence of on-going consumption has been the shortened survival of radiolabeled fibrinogen that improves after heparin administration (78), elevated plasma concentrations of fibrin by-products such as d-dimer (79) and fibrinopeptide A (80), and the inability to normalize factor levels after plasma infusions (81). On the other hand, many of the laboratory abnormalities suggestive of DIC such as thrombocytopenia, prolongation of PT, low fibrinogen levels, and elevated d-dimer concentration can occur in the setting of advanced liver failure even in the absence of on-going consumption (76). For instance, laboratory markers of fibrin degradation, such as d-dimer and fibrin split products, can be attributed to impaired hepatic clearance or accelerated fibrinolysis.

Accelerated fibrinolysis in cirrhosis was first described in 1914 by Goodpasture (82). He observed that blood samples taken at autopsy from four patients

with cirrhosis displayed rapid fibrin clot dissolution (hours) compared to the normal rate (days). He astutely concluded that "fibrinolysis is the activity of normal proteolytic ferments of the blood, operating by virtue of a diminuation or absence of the normal antiproteolytic substances." Abnormalities of the fibrinolytic pathway in advanced liver disease have since been attributed to a delay in hepatic clearance of plasmin activators such as tissue plasminogen activator (83,84) or to decreased synthesis of plasmin inhibitors including plaminogen activator inhibitor-1 (83,85,86), α_2-antiplasmin (84,87), and thrombin-activatable fibrinolysis inhibitor (88,89). Ascitic fluid also contains fibrinolytic activity, and its re-entry into circulation is a postulated mechanism underlying systemic hyperfibrinolysis (90). Factor XIII is also necessary for fibrin polymer cross-linking and its levels may be reduced in patients with cirrhosis (91,92). Distinction between consumptive coagulopathy and hyperfibrinolysis is difficult in advanced liver disease but may be clinically relevant because the treatment with an antifibrinolytic agent can be therapeutically useful in one circumstance and relatively contraindicated in the other. Moreover, in a study of 112 patients with cirrhosis and varices but without a history of upper intestinal bleeding, the only clinical or laboratory variable that predicted an initial bleeding episode was hyperfibrinolysis (93).

Clinical and Laboratory Evaluation of Coagulopathy

Bleeding is a major complication and one of the principal causes of death in patients with cirrhosis (94,95). Although prothrombin activity is a component of the Child-Pugh's scoring system and reflects generalized liver dysfunction, coagulopathy itself is not an independent risk factor for variceal bleeding (96,97). In situations in which bleeding is not attributed to the vascular sequelae of portal hypertension, bleeding symptomatology may be helpful in identifying the underlying hemostatic defect. Mucosal sites of bleeding such as epistaxis or bleeding gums indicate an abnormality involving primary hemostasis due to either qualitative or quantitative platelet deficiencies. Soft tissue hematomas or intracranial hemorrhage may reflect inefficient fibrin clot deposition due to either factor deficiencies or accelerated fibrinolysis. Generalized oozing at venipuncture sites often heralds the development of a more severe coagulopathy such as DIC or severe hypofibrinogemia.



▪ **Figure 12.2** Multifactorial basis of coagulopathy in patients with advanced liver disease. DIC, disseminated intravascular coagulation.

Laboratory evidence of coagulopathy is common in patients with advanced liver disease. However, in the absence of bleeding or anticipated invasive procedure, corrective measures are usually not indicated because the risk of spontaneous bleeding does not correlate with the degree of PT or PTT prolongation (93,98). In fact, thrombosis is not infrequent in patients with advanced liver disease, and a patient should not be considered therapeutically "auto-anticoagulated" in this setting (99). The hemostatic basis for thrombosis in this setting is often multifactorial, including decreased synthesis of natural anticoagulants such as antithrombin, protein C, and protein S; decreased hepatic clearance of activated coagulation factors; or, possibly, high levels of circulating factor VIII. There is also evidence that levels of von Willebrand's factor protease are decreased in patients with cirrhosis, which may predispose patients to microangiopathic thrombosis, but this association has not been established (100).

The differentiation of the various causes of coagulopathy in patients with advanced liver disease is often complicated by the presence of more than one hemostatic abnormality (Fig. 12.2 and Table 12.1). An isolated elevation of PT may be the initial laboratory

abnormality to reflect severe hepatic dysfunction. Prolongation of both PT and PTT can be seen in deficiencies (functional or antigenic) of common pathway coagulation factors, dysfibrinogenemia or low fibrinogen, and/or DIC. Thrombin time (TT) can be used to diagnose dysfibrinogenemia, but prolongation also occurs in the setting of low levels of fibrinogen or elevated levels of fibrin split products, which interfere with fibrin polymerization. Hyperfibrinolysis is characterized by a shortened euglobulin clot lysis time, but this assay is most accurately performed in specialized laboratories. Measurement of factor V activity may be useful in differentiating between decreased hepatic factor synthesis and vitamin K deficiency (either dietary or a functional) because factor V does not require vitamin K–mediated γ-carboxylation. The diagnosis of DIC is often difficult in patients with advanced liver disease because many of the diagnostic laboratory parameters (i.e., elevated d-dimer level, fibrin split products, hypofibrinogenemia, thrombocytopenia) can be attributed to other pathologic processes or delayed clearance of activated coagulation factors. Factor VIII levels may be helpful in distinguishing between synthetic factor deficiencies and consumption. As previously discussed, endothelial cells also synthesize factor VIII, and therefore, its levels can be elevated even in advanced liver disease. Similarly, the onset of coagulopathy can

provide insight into the underlying etiology because a fairly rapid time course (days to weeks) of hemostatic abnormalities supports the diagnosis of DIC over chronic liver disease.

Table 12.1. Coagulation Assay Characteristics of Coagulopathies Associated with Liver Disease

Condition	PT	PTT	TT	Clot lysis time
Factor VII deficiency	+	-	-	-
Factor II, V, X deficiencies	+	±	-	-
Dysfibrinogen or hypofibrinogen	+	±	+	±
Hyperfibrinolysis	-	-	-	+
Disseminated intravascular coagulation	±	±	±	-

+, abnormal; -, normal values; PT, prothrombin time; PTT, partial thromboplastin time; TT, thrombin time.

Correction of Coagulopathy in Bleeding Patients

Fresh frozen plasma

Fresh frozen plasma (FFP) is generated by ultracentrifugation of whole blood and can be stored up to 1 year at -18°C FFP contains all coagulation factors and can be used to replace deficient factors. A dose of 10 to 20 mLkg typically increases coagulation factors by 20% immediately after transfusion. But volume expansion often limits the total increase for any given coagulation factor to 20% to 30% above baseline (101). This is especially relevant in cases of variceal bleeding in which volume expansion could have the undesirable effect of increasing portal pressures (102).

Although FFP administration is useful in temporizing a hepatic coagulopathy, routine use of FFP in the absence of bleeding does not have demonstrated benefit (103). In the only randomized trial addressing this issue, 20 patients with paracetamol overdose were randomized to receive FFP versus no treatment. Prophylactic administration did not result in an improvement in bleeding outcomes (104). Other assessments of the relative benefit of FFP versus pathogen-reduced products also failed to demonstrate a benefit of one product over another in liver disease (103).

Cryoprecipitate

Cryoprecipitate is generated from FFP that is thawed at cold temperatures. It is enriched in high-molecular-weight proteins including fibrinogen, von Willebrand's factor, factor VIII, and factor XIII. It is most commonly used as replacement therapy for hypofibrinogenemia or dysfibrinogenemia and contains approximately 250 mg of fibrinogen per unit. In general, one unit of cryoprecipitate per 10 kg of body weight will increase plasma fibrinogen by 50 mg/dL. Transfusion with cryoprecipitate is

recommended in the bleeding patient when the plasma fibrinogen level falls below 80 to 100 mg/dL.

Prothrombin complex

Vitamin K–dependent factors (e.g., prothrombin; factors VII, XI, X; and protein C and S) can be concentrated using aluminum hydroxide or barium sulfate. However, these concentrates also contain small quantities of thrombogenic activated factors. Thrombotic complications are particularly frequent in patients with liver disease, which has limited the utility of prothrombin complex in this setting (105).

Antifibrinolytics

ε-Aminocaproic acid (EACA), aprotinin, and tranexamic acid are antifibrinolytic agents that inhibit plasminogen activation. They may have a role in managing hemostatic complications during liver transplantation but their use in cirrhosis is limited because of the difficulty in excluding DIC, which is a contraindication to therapy.

Recombinant factor VIIa

Activated VIIa binds to tissue factor, which in turn activates other coagulation factors including factors IX and X. Recombinant factor VIIa (rFVIIa) has been

used to treat bleeding patients in a variety of settings including hemophilia, cardiac surgery, trauma, and warfarin overdose. In advanced liver disease, potential applications include prophylaxis before liver biopsy, variceal bleeding, increased intracranial pressure, and liver transplantation. However, there are limited data to support the use of rFVIIa in these settings. Its potential role in liver biopsy and transplantation is discussed in the subsequent section.

A single large randomized trial investigated the potential benefit of rFVIIa in conjunction with endoscopic therapy for variceal bleeding (106). A total of 245 patients with cirrhosis complicated by upper gastrointestinal bleeding were randomized to rFVIIa versus placebo before endoscopy. rVIIIa failed to show any benefit in any endpoint including failure to control bleeding within the first 24 hours, prevention of rebleeding, early or late mortality, red cell requirements, or even active bleeding at first endoscopy. Therefore, the routine use of rFVIIa cannot be recommended in this setting. The administration of rFVIIa is currently being explored as rescue therapy in cases of uncontrolled variceal bleeding.

Coagulopathy Management in Liver Biopsy and Transplantation

Liver biopsy

Liver biopsy is common in the diagnostic evaluation of patients with liver disease. Following transcutaneous biopsy, intraperitoneal hemorrhage occurs in less than 5% of cases and the mortality rate is less than 0.5% (107,108,109,110,111). Mindful of the potential for bleeding complications, patients who are considered "high risk" on the basis of an abnormal coagulation profile, often receive FFP prophylactically. However, it is not clear from the literature that mildly abnormal hemostatic indices are predictive of bleeding outcomes in this patient population (107). In a prospective study of 200 consecutive patients who underwent laparoscopic biopsy and subsequent observation, there was no difference in terms of platelet count or PT among those patients who had prolonged bleeding at the biopsy site, as shown in Table 12.2 (110). Several retrospective studies have similarly failed to identify an association between PT and bleeding complications (108), although in one study the bleeding rates doubled in those patients with an INR equal to 1.5 or more compared to 1.3 to 1.5 (112). Other biopsy methods such as transjugular approach or biopsy site plugging with gelatin or

foam appear to have favorable bleeding outcomes in patients with coagulopathy but absolute indications for their use have not been established. On the basis of the available data, the British Society of Gastroenterology issued guidelines on the safety of percutaneous liver biopsy in patients with coagulopathy, as shown in Table 12.3 (113).

Table 12.2. Bleeding Outcomes in Patients with Abnormal Coagulation Profiles Undergoing Liver Biopsy

Study	Definition of major bleeding	Biopsy route	Bleeding with abnormal PT	Bleeding with normal PT
Ewe (107)	Directly visualized liver bleeding time. Major bleed >12 min	Lap	4/93 (4.3%)	4/85 (4.7%)
Riley (114)	Intraperitoneal bleeding	PC + plug	1/20 (5%)	NR
Tobin (115)	Decrease in hematocrit	PC + plug	1/100 (1%)	NR
McVay (116)	Hemoglobin decrease by 2 g/dL	PC	4/65 mild coagulopathy; 0/11 moderate (5.3% total)	4/100 (4%)
Papatheodoridis (117)	Requiring transfusion	TJ	0/112	0/45
Bruzzi (118)	Undefined	TJ	0/31	0/19
Smith (119)	Intraperitoneal hemorrhage detected clinically and by computed tomography	TJ	3/203 (1.5%)	0/168

PT, prothrombin time; Lap, laparoscopic biopsy; PC, percutaneous; NR, not recorded; TJ, transjugular.
Table adapted from Segal JB, Dzik WH. Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. *Transfusion* 2005;45(9):1413–1425 (120).

Orthotopic liver transplantation

Bleeding complications are frequent in liver transplantation and directly correlate with the morbidity and mortality of the procedure (121). The mechanisms underlying coagulopathy of liver transplantation can be divided into three surgical stages:

Preanhepatic, anhepatic, and postanhepatic stages.

In the *preanhepatic* stage of transplantation, the liver is surgically isolated. Bleeding during this stage may correlate with underlying coagulopathy, portal hypertension, and complexity of surgical intervention (122,123), although other analyses have failed to even

identify coagulopathy as a risk factor for intraoperative blood loss (124). Bleeding during *anhepatic* stage is characterized by oozing in the previously dry surgical bed. Laboratory findings suggestive of DIC have been demonstrated in earlier series but later studies have not confirmed significant changes in PTT or PT before reperfusion of the donor graft, which may be due to improved surgical techniques (125,126,127,128). Hyperfibrinolysis during the anhepatic stage is well documented and appears to correlate with an increase in circulating tissue-type plasminogen activator (t-PA) levels in the absence of hepatic clearance (129). Accelerated fibrinolysis also frequently complicates the postanhepatic stage, which has led to the evaluation of antifibrinolytic agents in liver transplantation.

Table 12.3. British Society of Gastroenterology Guidelines on the Management of Patients with Coagulopathy Requiring Percutaneous Liver Biopsy

British Society of Gastroenterology guidelines for percutaneous liver biopsy

- Platelet count and prothrombin time should be checked in the week prior, provided that the patient's liver disease is stable.
- If platelet count is >60,000/mm^3 then the biopsy can be safely performed. If the platelet count is 40,000–60,000/mm^3 then platelet transfusion may increase the count enough for the biopsy to be performed safely by the percutaneous route. If, however, platelet transfusion does not increase or the platelet count is <40,000/mm^3 then alternative biopsy methods such as plugged, transvenous (transjugular), or laparoscopic liver biopsy can be tried (*Recommendation grade B*).
- If the prothrombin time is <4 s prolonged, then percutaneous biopsy can be safely undertaken. If the prothrombin time is 4–6 s prolonged then a transfusion of fresh frozen plasma may bring the prothrombin time into the desired range. If the prothrombin time is >6 s prolonged then other biopsy methods should be tried (*Recommendation grade B*).
- Post liver biopsy observation should continue for 6 h, and if at the end of this period there have been no complications then the patient may be discharged. The patient should, however, have a responsible person to stay with on the first postbiopsy night and should be able to return to hospital within 30 min should the need arise (*Recommendation grade B*).

Definition of Grade B recommendation: Requires the availability of clinical studies without randomization.

Grant A, Neuberger J. Guidelines on the use of liver biopsy in clinical practice. British Society of Gastroenterology. *Gut* 1999;45(Suppl 4):IV1–IV11.

Aprotinin has been evaluated in 137 patients who underwent orthotopic transplantation (130,131). Patients were randomized to high-dose or regular-dose aprotinin versus

placebo. Intraoperative blood loss was significantly lower (44% to 60%) in the aprotinin-treated patients, which was reflected in a decreased number of red blood cell transfusions. However, differences in postoperative mortality were not observed. Other antifibrinolytic agents appear to have similar efficacy in liver transplantation (132,133,134). There have been several case reports of thrombosis after administration of aprotinin in liver transplantation (135), which has led some centers to question its empiric use (131). However, on the basis of the data taken from prospective randomized trials, the observed thrombosis rate does not appear to differ appreciably from the placebo-treated patients (Table 12.4) (136).

Table 12.4. Thrombosis Rates in Randomized Trials Using Antifibrinolytic Agents in Liver Transplantation

Antifibrinolytic agent	Number of patients	Hepatic artery thrombosis	Overall thrombosis
Aprotinin	493	3 (0.6%)	8 (1.6%)
Tranexamic Acid	157	7 (4.5%)	9 (5.7%)
ε-Aminocaproic acid	42	2 (4.8%)	2 (4.8%)
Placebo	264	7 (2.7%)	7 (2.7%)

Adapted from Porte RJ. Antifibrinolytics in liver transplantation: they are effective, but what about the risk-benefit ratio? *Liver Transpl* 2004;10:(2):285–288.

rFVIIa was recently evaluated in a prospective, randomized trial for patients undergoing liver resection in noncirrhotic patients (137,138). Two hundred and four patients were randomized to either 20 or 80 μg/kg of rFVIIa versus placebo. Perioperative red blood cell transfusion and intraoperative blood loss was not significantly reduced in the rFVIIa-treated patients. Similar findings were shown in two randomized, placebo-controlled trials of patients undergoing orthotopic liver transplantation (107). The number of RBC units transfused and intraoperative blood loss was similar in the rVIIa- and placebo-treated groups. On the basis of these randomized studies, the routine use of rVIIa in hepatic transplantation cannot be recommended.

Annotated References

Banerjee S, Owen C, Chopra S. Sickle cell hepatopathy. *Hepatology* 2001;33:1021–1028.

This paper is a comprehensive review of hepatobiliary complications accompanying sickle cell disease (SCD), including acute sickle hepatic crisis, hepatic sequestration crisis, sickle cell intrahepatic cholestasis, cholelithiasis, and multitransfusion hepatopathy from iron overload and viral hepatitis. The authors review the role of abdominal imaging studies in distinguishing the various pathophysiologic processes impacting liver function in patients with SCD, findings on liver biopsy, and the effects on the liver of treatments used in managing patients with SCD, including hydroxyurea,

bone marrow transplantation, and liver transplantation.

Blanchard RA, Furie BC, Jorgensen M, Kruger SF, Furie B. Acquired vitamin K-dependent carboxylation deficiency in liver disease. *N Engl J Med* 1981;305:242–248.

This is the initial description of impaired carboxylation of prothrombin present in over 90% of patients with hepatic disease.

Bosch J, Thabut D, Bendtsen F, et al. Recombinant factor VIIa for upper gastrointestinal bleeding in patients with cirrhosis: a randomized, double-blind trial. *Gastroenterology* 2004;127:1123–1130.

A total of 245 patients with upper GI bleeding were prospectively randomized to eight doses of FVIIa or placebo. There was no difference between the two groups in terms of control of bleeding after the first dose of FVIIa, failure to prevent rebleeding, or 5-day mortality.

Chitale AA, Sterling RK, Post AB, Silver BJ, Mulligen DC, Schulak JA. Resolution of spur cell anemia with liver transplantation: a case report and review of the literature. *Transplantation* 1998;65:993–995.

This paper describes a young patient with hemochromatosis, alcohol abuse, decompensated cirrhosis, and spur cell anemia whose spur cell anemia persisted after portal decompression by transjugular intrahepatic portosystemic shunt for recurrent bleeding esophageal varices. However, spur cell anemia resolved after orthotopic liver transplantation, suggesting an integral role for diseased liver, rather than spleen, in the development of red blood cell membrane changes accompanying liver disease.

Peck-Radosavljevic M, Wichlas M, Zacherl J, et al. Thrombopoietin induces rapid resolution of thrombocytopenia after orthotopic liver transplantation through increased platelet production. *Blood* 2000;95:795–801.

To clarify the contributions of platelet production, platelet consumption, coagulation activation, and splenic sequestration to thrombocytopenia in liver disease, the authors studied thrombopoietin (TPO) serum levels and markers of platelet production, platelet activation, and coagulation activation before and 14 days after orthotopic liver transplantation (OLT) in 18 patients with advanced liver cirrhosis. With increased TPO serum levels and increased bone marrow production of platelets, platelet counts normalized in most patients within 14 days of OLT, irrespective of the change in spleen size assessed by computed tomography volumetry. This underscores decreased TPO production in the cirrhotic liver as an important etiologic factor for thrombocytopenia in liver disease.

Segal JB, Dzik WH. Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. *Transfusion* 2005;45:1413–1425.

This reference is a detailed analysis of the current literature on the bleeding rates associated with invasive procedures including liver biopsies. The authors conclude that there is insufficient evidence to suggest that an abnormal coagulation profile is predictive of bleeding outcomes.

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Chapter 13
Nutrition and the Liver

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Key Concepts

- Energy expenditure is close to that predicted in patients with acute hepatitis and stable cirrhosis but is increased with respect to lean body mass. Energy expenditure is also increased in patients with hepatocellular carcinoma and in those with ascites.
- Both protein synthesis and metabolism are impaired in patients with hepatic dysfunction. Specifically, visceral protein synthesis is decreased, and therefore, serum albumin and prealbumin are not useful in the assessment of nutritional status.
- Patients with cirrhosis exhibit peripheral insulin resistance and hyperglycemia, and even overt diabetes may develop.
- Protein-energy malnutrition (PEM) is common in patients with chronic liver disease and is often related to the severity of the underlying liver disease. Malnutrition is associated with increased infection risk, multiple organ complications, esophageal variceal hemorrhage, and increased post-transplantation morbidity and mortality.
- Vitamin A, D, and E deficiency may develop in patients with chronic cholestatic disease such as primary biliary cirrhosis.
- Many patients with metabolic syndrome also develop nonalcoholic steatohepatitis (NASH).
- Hepatic dysfunction associated with the use of parenteral nutrition may more likely be the result of malabsorption rather than direct toxicity from the parenteral nutrient solution. Choline deficiency has been invoked as a primary cause for the hepatic steatosis that develops, and experimental data suggests a second hit, such as that from endotoxin, may be necessary for the development of more progressive liver disease.
- Some patients with alcoholic hepatitis, as well as those with cirrhosis, may have improved morbidity and survival with the institution of either enteral or parenteral nutritional support.
- Protein restriction should not be considered routine in patients with cirrhosis because most patients will tolerate an increased amount of standard dietary protein without development of or worsening of encephalopathy. Portosystemic encephalopathy should be aggressively treated using standard therapy before instituting dietary protein restriction. Select patients with hepatic encephalopathy may benefit from use of high branched-chain/low aromatic amino acid formulas.
- Because of ineffective gluconeogenesis, a continuous glucose or dextrose supply should be provided to the patient with hepatic failure awaiting liver transplantation. Post-transplantation indications for nutritional support are similar to other nontransplantation postoperative indications.

The liver serves many critical metabolic and nutritional functions. It is the major site for (i) the synthesis of key plasma proteins, such as albumin and coagulation factors; (ii) urea synthesis needed for normal nitrogen and ammonia metabolism; (iii) glucose production to maintain glucose homeostasis and prevent hypoglycemia; (iv) lipoprotein production and export of water-insoluble triglycerides from the liver as water-soluble very low density lipoprotein (VLDL)–triglyceride particles into the bloodstream; (v) ketone body production, which provides fuel for the brain during energy or carbohydrate restriction; (vi) catabolism of metabolic regulatory hormones, such as insulin; and (vii) production of inflammatory markers, such as C-reactive protein. These diverse metabolic functions require a considerable amount of energy. Although the liver represents only approximately 2% of body weight, it accounts for approximately 20% of the body's resting energy requirements. Serious liver disease not only impairs hepatic function but also has considerable extrahepatic metabolic effects on glucose (insulin resistance and impaired glucose tolerance), lipid (increased lipolytic rates), and protein (decreased protein synthesis and increased amino acid oxidation rates) metabolism (1). Therefore, liver function, nutrition, and metabolism are integrally related, and abnormalities in one have adverse effects on the other.

Metabolic and Nutritional Principles

Energy Intake and Absorption

The average person ingests approximately 50 million calories, composed of approximately 2,000 kg of protein, 8,000 kg of carbohydrates, and 2,500 kg of fat, during his/her lifetime. Therefore, the balance between energy intake and energy expenditure is carefully regulated to permit normal growth during childhood and pregnancy and prevent large changes in body composition during adulthood. Food intake is controlled by a complex interaction of peripheral and central systems that determines the size, content, and frequency of feedings (2). These regulatory mechanisms must integrate daily short-term bursts of energy intake (meals and snacks) with long-term energy requirements and body composition. The regulation of energy intake is further complicated by the influence of external environmental cues, which can override endogenous regulatory signals and cause under- or overconsumption of energy.

Patients with cirrhosis often experience alterations in nutrient intake, which can lead to weight loss. Several mechanisms contribute to decreased food intake, including decreased sensation to salty, bitter, sweet, and sour tastes; dysgeusia due to vitamin A or zinc deficiency (3,4); early satiety associated with ascites; medication-induced anorexia or nausea; and psychological or neurologic impairment that alter eating behavior (5). Alcohol intake can account for more than 50% of daily energy intake in some patients with alcoholic liver disease. Excessive alcohol is metabolized in part by the microsomal ethanol oxidizing system, which generates a wasteful energy cycle by producing heat without energy production (6).

Malabsorption can also contribute to weight loss in patients with liver disease, even when appetite is unchanged. Chronic cholestasis and decreased bile acid secretion reduces the formation of mixed micelles, which impairs fat (7,8) and fat-soluble vitamin absorption (9). Malabsorption can also be caused by lactulose therapy, which can exacerbate steatorrhea (8), and by small bowel bacterial overgrowth (10,11,12,13), which occurs because of impaired small bowel motility (14,15,16,17) and prolonged intestinal transit (11,18,19). Some patients with alcoholic liver disease have serious malabsorption because of pancreatic insufficiency or direct alcohol-induced injury of the small intestine.

Energy Metabolism

Energy is constantly required for normal organ function, metabolism, heat production, and muscular work. Resting energy expenditure (REE) is the amount of energy consumed during postabsorptive resting conditions and represents approximately 70% of total daily energy expenditure (TDEE). The thermic effect of feeding is the amount of energy expended during consumption and processing of ingested food and represents approximately 10% of TDEE. The energy expenditure of physical activity depends on the intensity and duration of daily activities but normally represents approximately 20% of TDEE.

An assessment of energy requirements is necessary to make appropriate decisions about dietary intake and nutritional therapy in patients with liver disease. The Harris-Benedict equation developed in 1919 (20) is

still a useful tool for estimating REE in adult men and women:

Men: REE = 66 + (13.7 × W) + (5 × H) - (6.8 × A)

Women: REE = 665 + (9.6 × W) + (1.8 × H) - (4.7 × A)

where *W*, weight in kg; *H*, height in cm; *A*, age in years.

The Harris-Benedict equation generates estimates of REE that are usually within 10% to 15% of measured values for healthy subjects (21). However, the values are less reliable in obese (tends to overestimate REE) or very lean (tends to underestimate REE) patients. In addition, hypocaloric feeding decreases REE to values that are 15% to 20% below those expected for actual body size, whereas metabolic stress, such as inflammatory diseases or trauma, increase energy requirements.

The effect of liver disease on energy requirements is confusing because of conflicting data from different studies. Patients with liver disease have been reported to have low, normal, and high resting metabolic rates (14,22,23,24). Part of the inconsistency across studies can be attributed to how the data are expressed and the clinical condition of the patient. For example, in patients with either acute hepatitis or stable cirrhosis, energy expenditure is normal when it is expressed with respect to body surface area but is increased when it is expressed with respect to lean body mass (15,25). Energy expenditure is also increased in patients who have hepatocellular carcinoma, in relation to tumor size (26,27). Patients with severe disease, such as those with acute hepatic failure, usually have a marked increase in energy expenditure (28,29). Energy expenditure is increased in patients with ascites and decreases after ascites is removed by paracentesis (30).

Energy expenditure can be determined by indirect calorimetry, which measures whole body oxidation of fuels. This is usually performed by using a metabolic cart to assess oxygen consumption and carbon dioxide production and can be helpful in certain patients, such as those with increased energy requirements (e.g., closed head injury, burn, and trauma), decreased energy requirements (e.g., cachexia), difficulty weaning from mechanical ventilation, and fluid overload, making it difficult to determine dry weight. However, the values obtained by indirect calorimetry require an experienced operator and carefully calibrated equipment used under appropriate conditions to obtain reliable results. Indirect calorimetry can be used to estimate basal energy expenditure, derived from oxygen and carbon dioxide consumption using the Weir equation [basal energy expenditure (kcal/day) = (3.941 × VO_2 [L/day]) + (1.106 × VCO_2 [L/day])] (31). Because oxygen phosphorylation allows continuous adenosine triphosphate (ATP) synthesis at the respiratory chain level, a close relationship exists between energy metabolism and oxygen consumption. The rate of ATP utilization determines the overall rate of substrate oxidation, and therefore, oxygen consumption. Indirect calorimetry is performed over a 20- to 30-minute period while the patient is at rest, but not sleeping; extrapolation to daily energy expenditure is undertaken. Indirect calorimetry is portable and noninvasive, and may be performed on spontaneously breathing or mechanically ventilated (FIO_2 60%) patients (Figs. 13.1A and B). In patients with cirrhosis, the liver comprises 20% to 30% of the whole body energy expenditure. Hypo- or hypermetabolism occur when the estimated energy expenditure varies by more than 10% of that predicted on the basis of the Harris-Benedict equation (20).

Data from studies that evaluated substrate oxidation by using indirect calorimetry suggests that cirrhosis shifts basal fuel use from carbohydrate to fat oxidation, presumably because of diminished hepatic glycogen stores and hepatic glucose production (15,16,25). In general, stable patients with liver disease or cirrhosis require approximately 30 kcal/kg per day.

Starvation

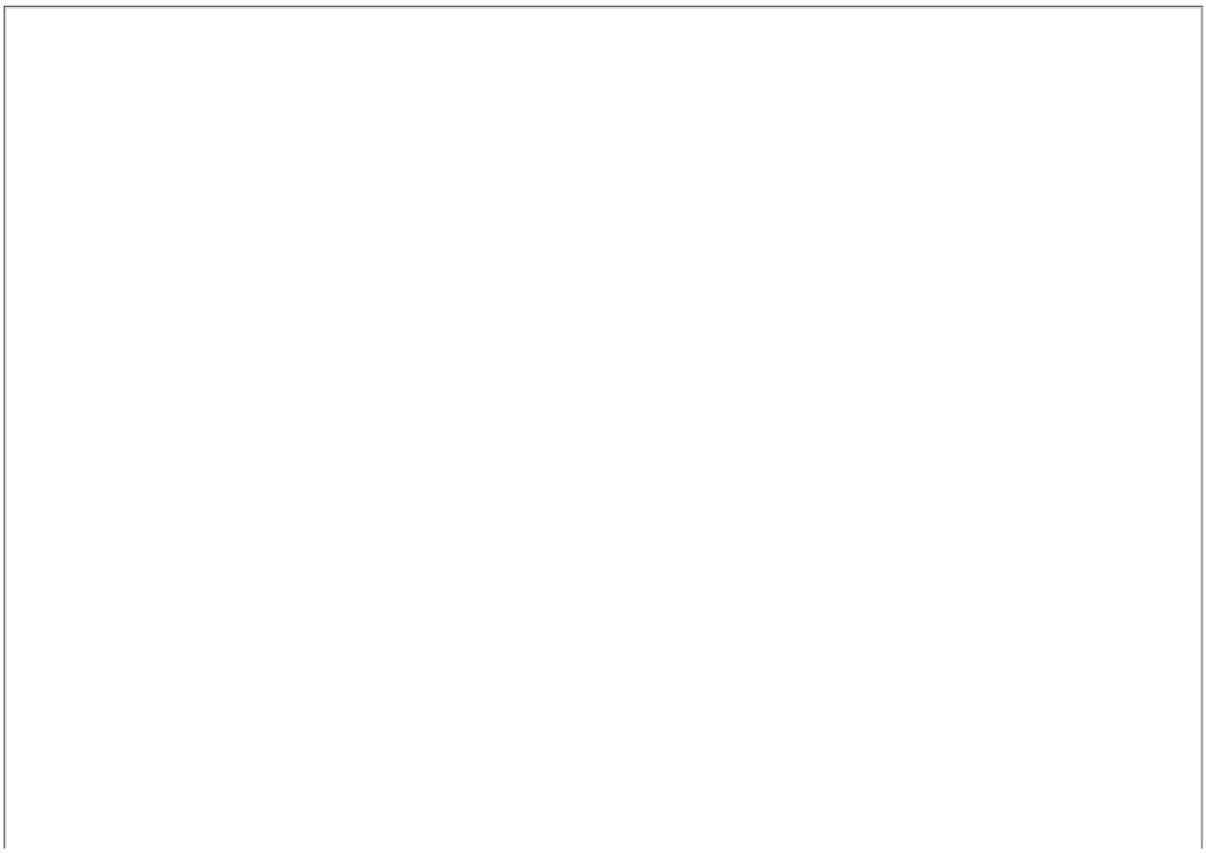
During starvation, an integrated series of metabolic adaptations occur that require important metabolic alterations for survival, including hepatic glycogenolysis and gluconeogenesis to maintain plasma glucose concentrations, decreased urea production in the liver to conserve body nitrogen, increased lipolysis and fatty acid release into plasma to provide tissues with fuel, and conversion of fatty acids to ketone bodies in the liver to provide a source of fuel for the brain and spare oxidative glucose requirements. Patients with cirrhosis have accelerated metabolic response to short-term starvation, manifested by a greater decline in hepatic glucose production, presumably related to decreased hepatic glycogen, and increased lipolysis of adipose tissue triglycerides (32).

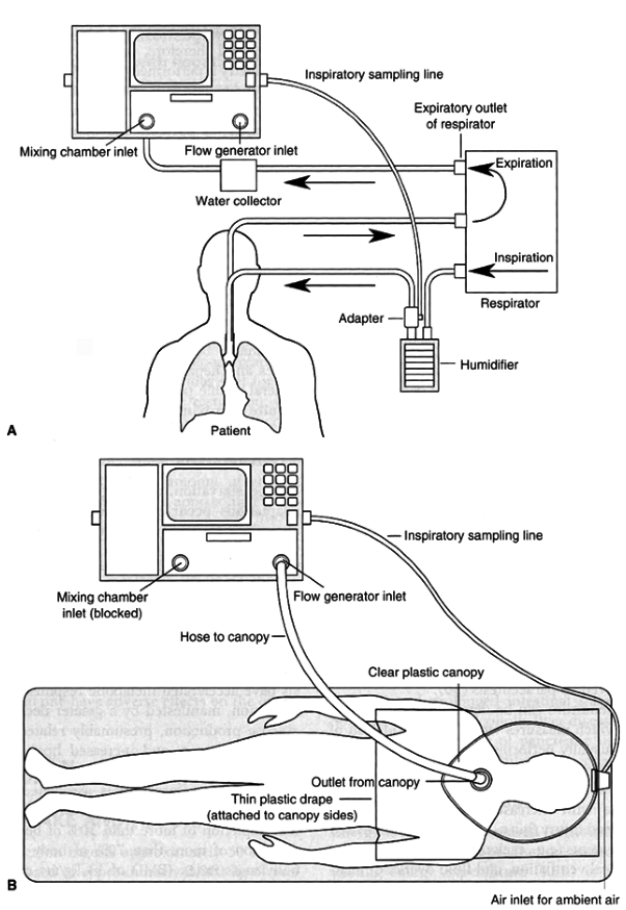
Death from starvation is associated with a body weight loss of more than 35% of body weight, protein depletion of more than 30% of body protein, fat depletion of more than 70% of body fat stores, and body mass index (BMI) of 13 kg/m^2 or less for men and 11 kg/m^2or less for women (33,34,35). Therefore, the duration of survival during starvation depends on the amount of available body fat and lean tissue mass and appropriate hepatic adaptations, which are compromised in patients with liver disease.

Protein Metabolism

Proteins are composed of a combination of 20 different amino acids, which are considered to be essential because the body cannot synthesize the carbon skeletons. Other amino acids are nonessential because they can be made from endogenous compounds. In some disease states,

nonessential amino acids may become "conditionally essential." For example, cysteine is essential in patients with cirrhosis because the trans-sulfuration pathway, which provides a sulfur group from methionine to cysteine, is impaired (36). The defect in trans-sulfuration affects methionine metabolism, which can lead to hypermethioninemia (37,38,39,40,41,42).





· Figure 13.1 A: Indirect calorimetry in a spontaneously breathing patient. **B:** Indirect calorimetry in a mechanically ventilated patient. (Reprinted with permission from Buchman AL. *Practical nutritional support techniques*, 2nd ed. Thorofare, NJ: Slack Inc., 2004.)

The liver is an important site for amino acid and ammonia metabolism. Transamination reactions are catalyzed by transaminases or aminotransferases (e.g., alanine transaminase [ALT] and aspartate transaminase [AST]), which interconvert a pair of amino acids with a pair of keto acids. An increase in serum transaminase concentration is a sign of liver injury and represents its

leakage from injured hepatocytes into the bloodstream. Ammonia, which is toxic to the central nervous system, is formed by oxidative deamination of glutamate in the liver, amino acid deamination in the kidneys, and intestinal bacterial production, and is absorbed and delivered to the liver through the portal vein. The liver rapidly removes ammonia from the circulation by formation of glutamate, glutamine, and urea. However, these metabolic processes can be impaired in patients with cirrhosis, causing an increase in circulating ammonia, which may contribute to hepatic encephalopathy (43).

A major function of the liver is to synthesize and secrete proteins that are necessary for normal metabolic function into the systemic circulation. Hepatic synthesis of albumin, which is necessary for maintaining normal intravascular oncotic pressure and transporting nutrients, and the synthesis of fibrinogen and clotting factors, needed for normal coagulation, are impaired in patients with advanced cirrhosis (44,45). In addition, steatohepatitis is associated with a lower rate of hepatic VLDL-apolipoprotein B production than in obese patients without steatohepatitis (46).

Muscle wasting occurs frequently in patients with cirrhosis (47). Skeletal muscle protein breakdown is often accelerated and nonoxidative leucine disposal is decreased in patients with cirrhosis (48). These alterations in amino acid metabolism can contribute to muscle wasting by enhancing protein breakdown in conjunction with diminished availability of amino acids for muscle protein synthesis.

Protein requirements are affected by the amount of nonprotein calories consumed, overall energy requirements, and protein quality. Inadequate amounts of any of the essential amino acids result in inefficient utilization. In general, approximately 15% to 20% of total protein requirements should be in the form of essential amino acids in healthy adults. The metabolic stress of illness often increases protein requirements by increasing protein catabolism and metabolic rate. Therefore, daily protein requirements in patients with serious liver disease who do not have encephalopathy (approximately 1.0 to 1.2 g/kg) may be greater than the protein requirements in the healthy adult population (approximately 0.8 g/kg) (Table 13.1) (49).

Nitrogen balance is calculated as the difference between nitrogen intake, in the form of amino acids or protein, and nitrogen losses in urine, stool, skin, body fluids, and nonprotein nitrogen. Nitrogen balance can be used to estimate protein balance because approximately 16% of proteins consists of nitrogen, and it is assumed that all the body nitrogen is incorporated into proteins. A positive balance (intake greater than losses) represents anabolic conditions and a net increase in total body protein content, whereas a negative balance demonstrates net protein catabolism. For example, a negative nitrogen balance of 1 g/day represents a 6.25-g/day (16% of 6.25 g protein = 1 g nitrogen) loss of body protein, which is equivalent to a 30-g/day loss of hydrated lean tissue. Total dietary nitrogen intake (grams) can be calculated by dividing the total protein intake (grams) over a 24-hour period by 6.25. Total urinary nitrogen (TUN), or urine urea nitrogen (UUN) + 4 to account for nonurea nitrogen losses (collected over the same 24-hour period as dietary intake), is then subtracted from the total nitrogen intake to arrive at the nitrogen balance. In practice, nitrogen balance studies tend to be artificially positive because of overestimation of dietary nitrogen intake and underestimation of losses caused by incomplete urine collections and unmeasured outputs.

Table 13.1. Recommended Daily Protein Intake

Clinical condition	Protein requirements (g/kg ideal body weight/d)

Normal	0.75
Metabolic "stress"[a]	1.0–1.5
Hemodialysis	1.2–1.4
Peritoneal dialysis	1.3–1.5
Continuous dialysis	1.7–2.0

[a]Including patients with liver disease who have encephalopathy.
Additional protein requirements are needed to compensate for excess protein loss in patients with burn injuries, open wounds, and protein-losing enteropathy or nephropathy. Lower protein intake may be necessary in patients with chronic renal insufficiency not treated by dialysis and in patients with liver disease and hepatic encephalopathy.

It is important to consider the patient's nutritional status when interpreting nitrogen balance data. When a person ingesting a low-protein diet is refed protein, nitrogen excretion does not rise proportionately to intake and there is retention of administered nitrogen. This gain during early refeeding is caused by a rapid accumulation of nitrogen in the liver and to a lesser extent in kidneys and muscle. However, the early retention of nitrogen is not sustained and nitrogen content decreases markedly within 4 to 7 days. In contrast, when a person ingesting a high-protein diet decreases his or her protein intake, the previously high urinary nitrogen loss continues for a few days despite the reduced intake, resulting in a negative nitrogen balance. Similarly, initial nitrogen loss after injury is greater in well-nourished than in malnourished patients. Therefore, a "labile" nitrogen pool of approximately 60 g contributes to short-term alterations in nitrogen balance. It must also be understood that a positive nitrogen balance requires a positive energy balance to avoid catabolism of skeletal muscle protein (50), although a positive nitrogen balance does not necessarily indicate adequate nitrogen utilization because protein metabolism is impaired in cirrhosis and end-stage liver disease.

Glucose Metabolism

After a person fasts overnight (12 hours), the liver produces approximately 2 mg of glucose/kg body weight (51). Adequate hepatic glucose production is critical because certain tissues, such as bone marrow, erythrocytes, leukocytes, renal medulla, eye tissues, and peripheral nerves, cannot metabolize fatty acids and require glucose (approximately 40 g/day) as a fuel, whereas other tissues, such as the brain, prefer glucose (approximately 120 g/day) as a fuel. Severe liver disease and cirrhosis can compromise the liver's capacity for glucose production (52).

Hepatic glucose production is an important determinant of basal plasma glucose concentrations. Therefore, impaired insulin-mediated suppression of endogenous hepatic glucose output can cause basal hyperglycemia and contribute to the development of diabetes. Excess fat in the liver (nonalcoholic fatty liver disease [NAFLD]) is associated with insulin resistance and features of the metabolic syndrome, including abdominal fat accumulation, diabetes, hypertriglyceridemia, low serum high-density lipoprotein (HDL) concentrations, and hypertension (53,54,55,56,57). Data from one study found that lean men with excessive intrahepatic fat content (approximately 11% of liver volume) had impaired insulin-mediated suppression of endogenous glucose production compared with lean men who had low liver fat content (approximately 2% of liver volume) (58). Moreover, moderate weight loss decreases intrahepatic fat content and normalizes hepatic insulin sensitivity (59).

The mechanisms underlying the relationships among hepatic fat, insulin resistance, and dyslipidemia are not known. It is possible that increased release of fatty acids from adipose tissue in obese persons simultaneously causes insulin resistance, dyslipidemia, and increased hepatic fat content. Excessive release of free fatty acids (FFAs) into plasma can impair the ability of insulin to stimulate muscle glucose uptake (60) and suppress hepatic glucose production (61,62) and can increase hepatic VLDL-triglyceride production and plasma triglyceride concentration (63). Therefore, increased hepatic fat content, insulin resistance, and dyslipidemia may be a sequelae of excessive fatty acid availability that can track together. However, it is also possible that increased hepatic fat itself has independent pathophysiologic effects by increasing intrahepatic fatty acids, which stimulate glucose production (61); fat metabolites, such as diacylglycerol and fatty acyl CoA; and ceramides, which impair insulin signaling in skeletal muscle (64).

Patients with cirrhosis exhibit resistance to insulin-mediated glucose metabolism in skeletal muscle. Despite normal endogenous hepatic glucose production (65,66,67), hyperglycemia or overt diabetes has been reported in 15% to 30% of patients with cirrhosis (68). Pancreatic β-cells are unable to secrete sufficient insulin to overcome peripheral insulin resistance (69), although prolonged hyperinsulinemia related to decreased insulin metabolism may actually account for the insulin resistance (70). Insulin resistance resolves after liver transplantation (71).

Lipid Metabolism

Lipids serve as a source of energy; structural components of cell membranes; precursors for steroid hormones, prostaglandins, thromboxane, and leukotriene synthesis; and carriers of essential nutrients. Plasma lipoproteins are molecular complexes of lipids and apolipoproteins that permit the transport of water-insoluble lipid species in the bloodstream. These complexes consist of a hydrophobic lipid core containing triglycerides and cholesterol esters with an outer surface of more hydrophilic phospholipids, free cholesterol, and apolipoproteins. One or more apolipoproteins are associated with each particle. Lipoproteins comprise a spectrum of particles of differing lipid content, apolipoproteins, and sizes and are commonly classified as chylomicrons, VLDL, low-density lipoprotein (LDL), and HDL on the basis of their floatation in an ultracentrifuge. VLDLs, produced by the liver, are the major endogenous triglyceride-rich lipoproteins. Obesity and NAFLD are associated with increased rates of hepatic VLDL-triglyceride production, which decrease with moderate weight loss (72).

Dietary lipids are composed mainly of triglycerides: Long-chain triglycerides (LCTs), which contain fatty acids that are more than 12 carbons in length, or medium-chain triglycerides (MCTs), which are 6 to 12 carbons in length. MCTs do not require bile acids for absorption and are released directly into the portal vein after intestinal absorption. The use of MCTs can be beneficial in patients who have disorders of fat digestion (e.g., pancreatic insufficiency, biliary obstruction), fat absorption (e.g., celiac sprue, short bowel syndrome), or lipid transport (e.g., intestinal lymphangiectasia, abetalipoproteinemia), or in those who require a reduction in lymphatic flow (e.g., chylous ascites, thoracic duct fistula) (73). However, MCTs are more ketogenic than LCTs and should not be given to patients with cirrhosis, particularly those with portal–systemic shunts.

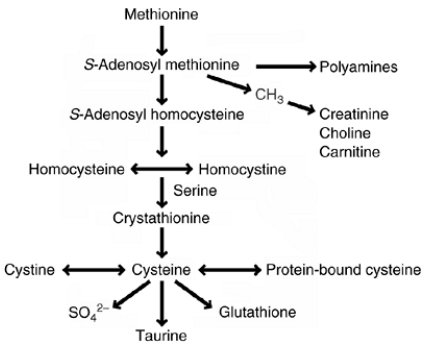
Major and Trace Minerals

Major minerals are inorganic nutrients that are required in large (>100 mg/day) quantities and are important for maintaining ionic equilibrium, water balance, and normal cell function. In patients with cirrhosis, excess whole body sodium exacerbates edema and ascites (74), and hypokalemia can precipitate encephalopathy (75).

Micronutrients

Micronutrients consist of trace elements and vitamins, which are constituents of enzyme complexes that regulate metabolic processes. Trace minerals are inorganic nutrients that are required in small (<100 mg/day) quantities. Normal intake of certain trace elements can be toxic to patients with cholestatic liver disease because these elements are excreted in the bile. For example, the recommended daily parenteral intake of manganese in patients with chronic liver disease can cause excessive manganese deposition in basal ganglia, causing extrapyramidal and Parkinson-like symptoms (76). Manganese should not be provided to patients with cholestasis. Copper intake should also be restricted in patients with cholestasis because it is primarily excreted through the biliary route, although no case of copper toxicity in patients who require parenteral nutrition has been described.

Choline is a particularly important micronutrient for the liver. Cirrhosis develops in the chronically choline-deficient rats (77), Rhesus monkeys (78,79), and Macaque monkeys (80). Hepatocellular carcinoma can also develop in a significant percentage of rats provided a chronic choline-deficient diet (81,82). In cirrhosis, choline deficiency may develop because of impaired methionine metabolism through the hepatic trans-sulfuration pathway (Fig. 13.2) (37,38,83). Hypermethioninemia develops and methionine plasma clearance is reduced after intravenous injection (39,42,83). Plasma free choline, glutathione, carnitine, taurine, and cysteine concentrations are decreased (84,85,86). Sulphate excretion is slowed in patients with cirrhosis; this also suggests impaired capacity of the trans-sulfuration pathway (41,42). The impairment in the trans-sulfuration pathway appears to be at the level of the trans-sulfuration of homocysteine to betaine (86). Ethanol also increases choline metabolism (87). Supplementation of the diet with choline in one study in monkeys prevented the development of hepatic fibrosis (88), although another study in baboons failed to confirm these findings (89); polyunsaturated lecithin and phosphatidylcholine (both containing choline) prevented the development of alcoholic fibrosis in the livers of baboons (90,91). Data from one study suggest that phosphatidylcholine supplementation decreases portal hypertension and ascites, improves response to hepatic encephalopathy, and decreases mortality compared with standard therapy alone (92).



· **Figure 13.2** The trans-sulfuration pathway.

Vitamins

Vitamins are organic compounds that are required in small (<100 mg/day) quantities. Body stores of water-soluble vitamins are usually much smaller than those of fat-soluble vitamins. Therefore, clinical symptoms of vitamin deficiencies usually occur more rapidly for water-soluble than for fat-soluble vitamins. Patients with liver disease are at particularly high risk for certain vitamin abnormalities.

B Vitamins

Patients with alcoholic liver disease have decreased serum concentrations of vitamins B_1 (thiamine), B_2 (riboflavin), and B_6(pyridoxine), and folic acid (93,94). Serum vitamin B_{12} concentration may be elevated because of its release from injured hepatocytes (93,94).

Vitamin A

Serum vitamin A concentration can be decreased in patients with cholestatic liver disease, such as primary biliary cirrhosis, and in patients with hepatocellular carcinoma (95,96). However, the decrease in serum concentration may be due to its defective mobilization from the liver rather than decreased intake or absorption (97). Low serum concentration of vitamin A can also be caused by decreased retinol-binding protein synthesis (98) and does not necessarily correlate with hepatic concentration (99). Plasma concentrations of retinol and retinyl esters decrease when hepatic stores are depleted. Vitamin A supplementation is essential in patients with alcoholic liver disease, in which dark adaptation difficulties are manifest. Abnormal dark adaptation or night blindness in conjunction with low serum vitamin A concentrations can be corrected by 4 to 12 weeks of treatment with 25,000 U of retinol per day (100). During replacement therapy, serum vitamin A concentrations should be monitored to avoid vitamin A toxicity, although it would take months or years of supplementation at this dose to cause toxicity in deficient patients (101). Both vitamin A and zinc deficiency are more prevalent in patients who

have portosystemic shunting for reasons that are not clear (102). Abnormal serum vitamin A concentrations in patients with severe liver disease often normalize within 2 weeks of liver transplantation (103).

Vitamin A toxicity, which is rare, is manifested by mild elevations in serum aminotransferase concentrations, hepatomegaly, dry skin, pruritus, brittle nails, hair loss, gingivitis, cheilosis, skeletal pain, pseudotumor cerebri, headache, anorexia, and fatigue. Serum retinol concentration is usually elevated but may initially be normal in some patients (104). The capacity of retinol-binding protein to transport vitamin A is exceeded, and the excess circulates as retinyl esters bound to plasma lipoproteins (105). Ito cells are the major storage site for retinyl esters (106). As such, these cells are increased in number in hypervitaminosis A (107) and can transform into fibroblasts, which produce type III collagen and fibrosis (108,109). Liver disease in patients with vitamin A toxicosis may take anywhere between 2 months and 1 year to resolve because of the prolonged half-life of vitamin A in the hepatic tissue (up to 60 days) (110,111,112,113,114,115,116). Discontinuation of vitamin A supplementation is the only effective therapy for vitamin A toxicity, but sometimes the liver disease progresses despite stopping vitamin A.

Vitamin D

Low serum 25-hydroxyvitamin D concentrations are observed in patients with chronic cholestasis, particularly primary biliary cirrhosis, because of vitamin D malabsorption (117,118). Patients with chronic liver disease may also fail to obtain sufficient sunlight exposure, which decreases vitamin D production (119). Vitamin D deficiency may also occur in patients with hemochromatosis, but blood levels of vitamin D increase after venesection therapy to remove excess iron (120). Patients with alcoholic cirrhosis have impaired hepatic 25-hydroxylation (121,122) and are at particularly high risk for osteoporosis, which can develop in approximately 50% of these patients (123).

Because vitamin D enhances intestinal calcium absorption (124), low concentrations of this vitamin are associated with decreased calcium absorption (123). Although supplementation with 25-hydroxyvitamin D increases calcium absorption, this formulation is no longer commercially available in the United States; oral 1,25-dihydroxyvitamin D is used instead (125). Vitamin D deficiency may persist after liver transplantation (126).

Decreased bone mineralization (osteomalacia) may occur because of vitamin D deficiency. Several case series have shown that osteomalacia developing in patients with primary biliary cirrhosis may be successfully treated with vitamin D supplementation (127,128,129). Bisphosphonate therapy may be useful in these patients. Other series, however, have reported that the prevalence of osteomalacia was extremely low but that the primary metabolic bone disease was osteoporosis, resulting from increased bone resorption (130,131,132). Data from one clinical study suggest that osteoporosis in some patients with primary biliary cirrhosis is unresponsive to vitamin D therapy and can progress to fractures (133,134). Other studies have shown that bone mineral density (BMD) increased in patients with alcoholic liver disease who were treated with large doses of vitamin D (50,000 IU/day of cholecalciferol or 25 to 50 mg/day of 25-hydroxyvitamin D) (135). Despite the use of medications such as prednisone, which has adverse effects on bone, liver transplantation increases BMD in children undergoing the procedure because of biliary atresia (136).

Vitamin E

Vitamin E deficiency has been described in patients with chronic cholestatic liver disease including primary biliary cirrhosis. Malabsorption appears to be the primary pathophysiology (137). The serum concentration, however, may not accurately reflect vitamin E status because this vitamin is primarily lipid bound. Therefore, vitamin E status should be determined from the ratio of serum vitamin E concentration to total serum lipid (i.e.,

cholesterol, triglycerides, phospholipids) concentration. A ratio of less than 0.5 mg/g or a serum vitamin E to total serum cholesterol concentration ratio of <235 µmol/mmol is considered evidence of deficiency (138,139). However, serum vitamin E to lipid ratio may not predict hepatic stores of the vitamin (140), although interestingly, serum vitamin E concentration alone correlates well with hepatic stores in patients with cirrhosis (141). Vitamin E deficiency may manifest in a neurologic syndrome that includes weakness and proximal muscle wasting, areflexia, and decreased proprioceptive and vibratory sensation (142) and hemolysis (143). Deficiency can be corrected with oral vitamin E supplementation of 400 to 1000 mg daily.

Vitamin K

Vitamin K deficiency is relatively uncommon in patients with cholestatic liver disease and when it occurs, it is usually found in conjunction with other fat-soluble vitamin deficiencies (e.g., vitamin A or E). Measurement of the prothrombin time is an insensitive indicator of vitamin K status and may often remain normal despite decreased plasma vitamin K_1 (phylloquinone) concentration (144). Vitamin K is a

cofactor for γ-glutamylcarboxylase, an enzyme that converts glutamate residues into γ-carboxyglutamate. Deficiency results in impaired carboxylation of prothrombin, producing abnormal prothrombin with no coagulant effect. Vitamin K deficiency has been associated with the development of metabolic bone disease, and supplementation with vitamin K_2 (vitamin form—menaquinone) leads to an increase in BMD in women with cirrhosis (145). This same study also suggested vitamin K_2 supplementation may have a preventive effect for hepatocellular carcinoma (146).

Trace Metals

Zinc

Zinc is required for the release of vitamin A from hepatic storage in retinol-binding proteins and therefore, zinc deficiency may promote vitamin A deficiency (147,148). Zinc supplementation may alleviate abnormalities in dark adaptation in some patients with cirrhosis (149). Zinc supplementation in patients with cirrhosis and low blood zinc concentration has been shown to increase the zinc concentration, improve urea kinetics, and alleviate encephalopathy (150). This finding suggests that low plasma zinc concentration is important in the pathophysiology of insulin resistance in cirrhosis and in improving glucose tolerance (151). Measurement of zinc in blood to detect deficiency may, however, be problematic. It is bound to albumin, and lower albumin concentration results in lower plasma zinc concentration (152); there is no known conversion factor to account for the low albumin level caused by decreased hepatic synthetic function. Therefore, reliance on plasma or serum zinc concentration may overestimate the prevalence of zinc deficiency in patients with significant hepatic dysfunction. Measurement of leukocyte zinc concentration may better reflect tissue stores (152). Increased renal zinc loss has been described in several studies in patients with either alcoholic-related or nonalcoholic liver disease and in patients with cirrhosis (153,154). In addition, intestinal zinc absorption may be impaired in some, but not all, patients with cirrhosis (155,156,157). After liver transplantation, serum zinc concentration increases and renal loss decreases in many patients (158).

Selenium

Serum hepatic selenium concentrations may be decreased in patients with cirrhosis, as well as in those with chronic hepatitis (159,160). Selenium deficiency has been associated with reversible cardiomyopathy, neuropathy, myopathy, macrocytosis, and pseudoalbinism. However, these have not been described in association with selenium deficiency in patients with liver disease (161,162). Burk et al. found that plasma glutathione peroxidase (GSH-Px) and selenoprotein P, two selenoproteins, were unaffected by low plasma selenium concentrations, suggesting that in patients with cirrhosis, low selenium concentration may itself not be a valid indicator of selenium deficiency (163), although other investigators have found both decreased plasma selenium and GSH-Px concentrations in patients with cirrhosis (164,165). Therefore, until selenium deficiency can be better defined in patients with hepatic dysfunction, routine supplementation cannot be recommended.

Malnutrition

Ingestion and absorption of a nutritionally adequate diet are necessary to maintain health. An imbalance between nutrient intake and requirements can lead to malnutrition, manifested by alterations in metabolism, organ function, and body composition. Malnutrition can be caused by specific mineral and micronutrient deficiencies. For example, inadequate iron consumption can cause iron deficiency and anemia, and inadequate vitamin D consumption can cause tetany, muscle weakness, and osteomalacia. Replacement of the deficient nutrient usually corrects the biochemical and functional abnormalities. However, prolonged micronutrient deficiency can cause irreversible organ damage. Malnutrition can also be caused by inadequate macronutrient intake. *Primary protein-energy malnutrition* (*PEM*) is caused by inadequate protein and energy consumption and usually affects children and elderly persons because of poor access to appropriate foods. Primary PEM includes several well-known deficiency syndromes described in children, such as kwashiorkor, marasmus, and nutritional dwarfism (Table 13.2). *Secondary PEM* represents fat and muscle depletion that

accompanies illness or injury and is caused by alterations in nutrient intake, digestion, absorption, or metabolism.

Table 13.2. Characteristics of Protein-Energy Malnutrition Syndromes in Children

Characteristic	Kwashiorkor	Marasmus	Nutritional dwarfism
Weight for age (% expected)	60–80	<60	<60
Weight for height	Normal or decreased	Markedly decreased	Normal
Edema	Present	Absent	Absent
Mood	Irritable when picked up	Apathetic when alone	Alert
Appetite	Poor	Good	Good

Specific micronutrient deficiencies and both primary and secondary PEM can affect liver structure and function. For example, choline deficiency causes hepatic steatosis (166). Both primary and secondary PEM can cause steatosis, abnormal liver biochemistries, hepatomegaly, and hepatic inflammation (167). In fact, abdominal distention observed in children with kwashiorkor is a consequence of hepatomegaly, in conjunction with intestinal dilatation and weak abdominal muscles. Ascites is not caused by PEM alone and is an indication that other pathogenic factors are involved. Hepatic steatosis is more marked when there is concomitant inflammatory stress, such as in children with kwashiorkor or patients with infection. For example, more than 40% of hepatocytes can have fatty infiltration in kwashiorkor (168). Increased liver biochemical test results occur in approximately 10% to 15% of patients who have anorexia nervosa (169). Serum alanine aminotransferase is the serum aminotransferase whose levels are most frequently elevated. Data from clinical reports have found that some patients with anorexia nervosa have serum aminotransaminase concentrations that are 20 times greater than the upper limits of the normal range. The mechanism responsible for the marked increase in liver biochemical test results is not clear. Histologic evaluation of liver tissue abnormalities in patients who have anorexia nervosa and abnormal liver biochemistries has detected a range of liver abnormalities, including subtle portal inflammatory infiltration to more severe ballooning of hepatocytes, accumulation of glycogen, and hepatic steatosis.

Nutrition support improves nutritional status when secondary PEM is mainly caused by anorexia and inadequate food intake. Although both structural and functional abnormalities associated with primary PEM are often reversible by nutritional therapy, prolonged primary PEM can cause irreversible changes in organ function and growth. Nutrition support is unlikely to restore muscle mass in patients who have acute or chronic inflammatory

stress because alterations in metabolism contribute to muscle wasting, which is unlikely to resolve until the underlying inflammatory illness is effectively treated.

Nutritional Assessment

Specific mineral and micronutrient deficiencies

Single mineral, vitamin, and trace mineral deficiencies often cause specific clinical abnormalities, which can be diagnosed by a comprehensive medical evaluation. Therefore, an appropriate history and physical examination, and laboratory tests can be used to diagnose specific nutrient deficiencies (Tables 13.3, 13.4 and 13.5) (170).

Protein-energy malnutrition

The diagnosis of PEM is different in adults compared with that in children because adults do not grow in height. In children, PEM results in growth and developmental delay. Therefore, pediatric growth charts are useful tools to help diagnose PEM in children because undernutrition stunts growth (170). The severity of PEM in children can be assessed by using the Waterlow classification, which determines a child's weight for height (wasting) and height for age (stunting) relative to normal median values (Table 13.6) (171).

Making a diagnosis of PEM in adults may be difficult because there is no universally accepted measure of nutritional status. All current parameters used to assess PEM are affected by illness and injury, so it is difficult to separate clinical influences from the influences of inadequate nutrient intake on markers of nutritional

status. In addition, nutritional assessment markers have been validated by determining their correlation with clinical outcome. Therefore, current nutritional assessment techniques identify patients who are at high risk for complications and mortality but do not necessarily identify patients who will benefit from nutritional therapy.

Table 13.3. Assessment of Mineral Deficiency

		Laboratory evaluation	
Mineral	**Symptoms or signs of deficiency**	**Test**	**Comment**
Sodium	Hypovolemia, weakness	Urinary sodium	May not represent body stores
Potassium	Weakness, paresthesias, arrhythmias	Serum potassium	May not represent body stores
Magnesium	Weakness, twitching, tetany, arrhythmias, hypocalcemia	Serum magnesium 24 h urine magnesium Urinary magnesium	May not represent body stores, urine is preferred May not represent body stores
Calcium	Osteomalacia, tetany, arrhythmias	Serum ionized calcium Dual-energy x-ray absorptiometry	Reflects bone calcium content
Phosphorous	Weakness, fatigue, hemolysis, respiratory muscle insufficiency	Plasma phosphorus	May not reflect body stores

Table 13.4. Assessment of Trace Mineral Deficiency

		Laboratory evaluation	
Trace Mineral	**Symptoms or signs of deficiency**	**Test**	**Comment**
Copper	Anemia, neutropenia, osteoporosis	Serum copper Plasma ceruloplasmin	May not reflect body stores Acute-phase reactant
Iodine	Hypothyroidism, goiter	Urine iodine Thyroid-stimulating hormone	Reflects recent intake Reflects thyroid function
Iron	Microcytic hypochromic anemia	Serum iron and total iron-binding capacity	Poor measure of body stores; high specificity when levels are low; poor sensitivity
Manganese[a]	Hypercholesterolemia, dementia, dermatitis	Serum manganese	May not reflect body stores
Selenium	Cardiomyopathy, myopathy, pseudoalbinism, macrocytosis	Serum selenium Blood glutathione peroxidase activity	May not reflect body stores
Zinc	Growth retardation, delayed sexual maturation, alopecia, acro-orificial skin lesion, diarrhea, mental status changes	Plasma zinc, leukocyte zinc	May not reflect body stores

[a]Manganese toxicity, manifested as extrapyramidal and parkinsonian-like symptoms, can occur in patients with chronic liver disease who are receiving long-term parenteral nutrition.

History and physical examination

We believe the best approach for evaluating nutritional status is to perform a careful *history and physical examination*. The history should assess

whether changes in body weight have recently occurred. Unintentional weight loss is associated with adverse clinical outcome. However, assessment of weight loss is complicated in patients with liver disease because of the confounding influence of edema and ascites on body weight. A loss of 10% or more of body weight within 6 months is associated with a poor clinical outcome (172). A diet assessment should be made to determine whether there has been a change in food intake, which can account for weight loss. Appetite, gastrointestinal function, and physical function should be reviewed to determine reasons for inadequate intake and whether alterations in nutrient intake were severe enough to lead to compromised function.

The physical examination should include a determination of BMI, which is calculated as weight (in kilogram) divided by height (in meter) squared. The assessment of BMI can help identify patients at increased risk for medical complications (Table 13.7). An estimate of "dry weight" in patients who have edema or ascites is necessary to obtain a valid estimate of the risk. Patients who are extremely underweight (BMI < 14 kg/m^2) are at high risk for nutrition-related medical complications and death and should be considered for hospital admission to receive nutritional therapy. The physical examination should search for evidence of fat and muscle wasting, decreased muscle strength, dehydration (e.g., hypotension, tachycardia, postural changes, mucosal xerosis, decreased axillary sweat, and dry skin), and fluid overload (e.g., edema, ascites).

Serum albumin concentration

In healthy adults, approximately 14 g of albumin is produced by the liver daily, which represents approximately 5% of the total 300 g body pool of albumin. Newly synthesized albumin has a half-life of approximately 20 days. Approximately one third of the total albumin pool is located in intravascular space and two thirds in extravascular space; approximately 5% of the intravascular albumin pool exchanges with extravascular albumin every hour, so there is a constant exchange of albumin between the two pools.

Serum albumin concentration has been used as a measure of nutritional status because children who have kwashiorkor have low albumin concentrations, and hypoalbuminemia is associated with an increase in medical complications and mortality in adults (173). Low serum concentrations of these proteins can help identify patients who are seriously ill. However, serum protein concentration is not a good marker of nutritional status because illness or injury, not malnutrition, is responsible for hypoalbuminemia in sick patients (174).

Inflammation and injury decrease albumin synthesis, increase albumin degradation, and increase albumin transcapillary losses from the plasma compartment. In fact, prolonged protein-energy restriction in patients with anorexia nervosa (175) or in human volunteers (176) causes a marked decrease in body weight, but not hypoalbuminemia. Although fasting or inadequate protein and energy intake decreases the rate of albumin synthesis, serum albumin concentration does not usually decline because of albumin's large body pool size (300 g) and slow turnover rate (20-day half-life), a compensatory decrease in albumin degradation, and the transfer of extravascular albumin to the intravascular compartment (177,178).

Table 13.5. Assessment of Vitamin Deficiency

		Laboratory evaluation	
Vitamin	**Symptoms or signs of deficiency**	**Test**	**Comment**
A (Retinol)	Night blindness, Bitot's spots, keratomalacia, follicular hyperkeratosis, xerosis	Serum retinol	Reflects recent intake and body stores
D (Ergocalciferol)	Rickets, osteomalacia, osteoporosis, bone pain, muscle weakness, tetany	Serum 25-hydroxyvitamin D	Reflects body stores
E (α-tocopherol)	Hemolysis, retinopathy, neuropathy	Serum tocopherol Serum tocopherol: total lipid ratio	Reflects body stores Ratio is preferred test
K (Phylloquinone)	Easy bruising and bleeding, abnormal clotting	Prothrombin time	Not specific for vitamin K
B_1 (Thiamine)	Beriberi, cardiac failure, Wernicke's encephalopathy, peripheral neuropathy, fatigue, ophthalmoplegia	RBC transketolase activity	Reflects body stores
B_2 (Riboflavin)	Cheilosis, sore tongue and mouth, eye irritation, seborrheic dermatitis	RBC glutathione reductase activity	Reflects body stores
B_3 (Niacin)	Pellagra (dermatitis, diarrhea, dementia), sore mouth and tongue	Urinary *N*-methyl-nicotinamide	Reflects recent intake
B_5 (Pantothenic acid)	Fatigue, weakness, paresthesias, tenderness of heels and feet	Urinary pantothenic acid	Reflects recent intake
B_6 (Pyridoxine)	Seborrheic dermatitis, cheilosis, glossitis, peripheral neuritis, convulsions, hypochromic anemia	Plasma pyridoxal phosphate	Reflects body stores
B_7 (Biotin)	Seborrheic dermatitis, alopecia, change in mental status, seizures, myalgia, hyperesthesia	Plasma biotin	Unknown
B_9 (Folic acid)	Megaloblastic anemia, glossitis, diarrhea	Serum folic acid; RBC folic acid	Reflects body stores and recent intake Reflects body stores
B_{12} (Cobalamin)	Megaloblastic anemia, paresthesias, decreased vibratory or position sense, ataxia, mental status changes, diarrhea	Serum cobalamin, methylmalonic acid Serum methylmalonic acid	Reflects body stores Tests functional block in enzyme
C (Ascorbic acid)	Scurvy, petechia, purpura, gingival inflammation and bleeding weakness, depression	Plasma ascorbic acid Leukocyte ascorbic acid	Reflects recent intake Reflects recent stores

RBC, red blood cells.

The relationship between serum albumin concentration and nutritional status is particularly confounded in patients with liver disease. It is likely that

low plasma albumin concentrations in patients with liver disease is primarily caused by inflammation, which increases transcapillary losses of albumin from intravascular to extravascular compartments (179), and by decreased albumin synthesis in select patients with advanced cirrhosis. In fact, many patients with cirrhosis have normal or high rates of albumin synthesis (180).

Table 13.6. Waterlow Classification of Protein-Energy Malnutrition in Children

	Normal	Mild	Moderate	Severe
Weight for height (wasting)				
Percent of median NCHS standard	90–110	80–89	70–79	<70
Standard deviation from the NCHS median	+*Z* to -*Z*	-1.1 *Z* to -2 *Z*	-2.1 *Z* to -3 *Z*	<-3 *Z*
Height for age (stunting)				
Percent of median NCHS standard	95–105	90–94	85–89	<85
Standard deviation from the NCHS median	+*Z* to –*Z*	–1.1 *Z* to –2 *Z*	–2.1 *Z* to –3 *Z*	<–3 *Z*

NCHS, National Center for Health Statistics; *Z*, 1 standard deviation.

Serum prealbumin concentration

Prealbumin, which is a component of the retinol-binding prealbumin complex and serves as a transport protein for thyroid hormones, has also been proposed as a measure of nutritional status. It has been hypothesized that prealbumin is a better marker of nutritional status than albumin because prealbumin has a much shorter half-life (2 to 3 days) than albumin (approximately 20 days). Prealbumin is synthesized by the liver and catabolized by the kidneys. Serum prealbumin concentration is an unreliable index of nutritional status in patients with liver disease because its serum concentrations decrease in patients with liver disease or inflammation and increase in patients with renal failure (181).

Creatinine–height index

The amount of creatinine excreted in urine provides an index of skeletal muscle mass (182,183) and has been used as a marker of nutritional status. Every day, a small portion (approximately 2%) of creatine, which is distributed primarily in skeletal muscle, is converted by an irreversible, nonenzymatic reaction to creatinine in the liver. Creatinine is subsequently excreted unchanged in urine. The creatinine–height index (CHI) is determined by measuring urinary creatinine excretion for 24 hours in relation to the patient's height while the patient is consuming a creatine- and creatinine-free diet. It is not known whether CHI is decreased in patients with liver disease because of decreased hepatic creatinine synthesis.

Table 13.7. Classification of Nutritional Status by Body Mass Index in Adults

Body mass index (kg/m^2)	Nutritional status
<16.0	Severely malnourished
16.0–16.9	Moderately malnourished
17.0–18.4	Mildly malnourished
18.5–24.9	Normal
25.0–29.9	Overweight
30.0–34.9	Obese (class I)
35.0–39.9	Obese (class II)
>40.0	Obese (class III)

Immune competence

Delayed cutaneous hypersensitivity (DCH) is altered by severe malnutrition, and patients suffering from severe PEM can become anergic. Up to 60% of patients with cirrhosis and more than 90% of patients with acute hepatic failure are anergic (184). However, a large number of clinical factors also influence DCH, making it a poor marker of malnutrition in sick patients with liver disease. Cirrhosis, hepatitis, infection, uremia, immunosuppressant therapy, and surgery impair DCH in the absence of malnutrition.

Malnutrition in Patients with Liver Disease

PEM is common in patients with chronic liver disease. However, the criteria used to diagnose PEM are affected by liver disease itself, so the presence and severity of malnutrition is often related to the clinical stage of liver disease (185,186). For example, a survey conducted in more than 1,400 patients with cirrhosis found that 20% of patients with Child-Pugh A cirrhosis and 60% of those with Child-Pugh C cirrhosis had PEM (186). PEM is a nearly universal finding in patients awaiting liver transplantation (187,188).

Although indicators of nutritional status do not necessarily reflect adequacy of nutrient intake, these indicators can be useful prognosticators of clinical outcome in patients with liver disease. PEM is associated with an increased risk of infections, multiple organ complications, esophageal variceal hemorrhage, and increased mortality before transplantation, and increased risk of infection, prolonged hospitalization, and mortality after transplantation (188,189,190,191,192,193,194,195,196). Muscle wasting in patients with alcoholic cirrhosis is associated with increased postoperative mortality (197). Malnourished patients undergoing liver transplantation are twice as likely to have treatment-refractory ascites and not as likely to survive as those who are not judged to be malnourished (185,188).

Metabolic Syndrome and Nonalcoholic Liver Disease

Metabolic syndrome represents a constellation of metabolic abnormalities that are risk factors for coronary heart disease. The notion of a metabolic syndrome was first considered by Vague more than 50 years ago, who noticed that an increase in upper body fat, which he called *masculine or android obesity*, was observed commonly in obese men and was associated with diabetes, coronary heart disease, and gout (198).

The metabolic syndrome concept continued to advance and was been defined by the National Cholesterol Education Program as having three or more of the following characteristics: (i) Abdominal obesity (waist circumference >102 cm in men and >88 cm in women), (ii) increased fasting blood glucose level (>100 mg/dL), (iii) increased serum triglyceride levels (≥150 mg/dL), (iv) low serum HDL-cholesterol concentration (<40 mg/dL in men and <50 mg/dL in women), and (v) increased blood pressure (≥130/85 mm Hg) (199). The prevalence of the metabolic syndrome in adults increases directly with age. In the United States, the prevalence of the metabolic syndrome is approximately 7% in those aged 20 to 29 years and increases to approximately 45% in those aged 60 years and older (200). Many obese patients with metabolic syndrome also have NAFLD (201,202), and it has been suggested that NAFLD should be considered a component of the metabolic syndrome (203). In fact, the presence of metabolic syndrome may predict concurrent NAFLD (204). The mechanism responsible for the close relationship between metabolic syndrome and NAFLD is not clear, but it likely involves increased release of FFAs into the bloodstream, which can cause increased hepatic fatty acid uptake and triglyceride accumulation, increased hepatic glucose production, impaired insulin-mediated skeletal muscle glucose uptake, and increased hepatic VLDL-triglyceride production (62,205,206,207). Moreover, increased amount of intrahepatic fat itself can impair hepatic insulin action (58) and stimulate VLDL-triglyceride secretion by providing a source of fatty acids for triglyceride production (208).

Weight loss is the cornerstone of therapy for patients with the metabolic syndrome and NAFLD. Moderate weight loss ameliorates the metabolic abnormalities associated with both the metabolic syndrome and NAFLD. The results from several studies suggest that a gradual loss of 10% or more of body weight can correct abnormal liver chemistries and decrease liver size, fat content, and some features of steatohepatitis (209,210,211). Gastrointestinal surgery is the most effective approach for achieving weight loss in extremely obese patients. The gastric bypass procedure is the most common bariatric surgical procedure performed in the United States, accounting for more than 70% of all bariatric procedures. Currently, more than 140,000 gastric bypass procedures are performed each year in the United States; on average, obese patients who undergo gastric bypass lose about two thirds of their excess weight or approximately 30% of their initial weight (212). However, rapid and marked loss after very low calorie diets (213) or fasting (214) has been associated with an increase in hepatic inflammation and fibrosis, and even liver failure.

Parenteral Nutrition–Associated Liver Disease

Two forms of PN-associated liver disease have been described, one in which cholestasis predominates and the other in which steatosis (both micro- and macrovesicular) predominates. The former is more prevalent in infants, especially those who have been delivered preterm, and the latter predominates in adults, although there is overlap. Cholestasis is associated with hepatocyte ballooning, Kupffer cell hyperplasia, bile duct plugging, and, in infants, extramedullary hematopoiesis. Initially, inflammation is generally minimal, with limited periportal lymphocyte infiltration, although this may progress to steatohepatitis with hepatocyte necrosis and pericellular fibrosis, bile duct hyperplasia and proliferation, and eventual progression to cirrhosis and hepatic failure.

Serum hepatic aminotransferase concentrations (i.e., AST, ALT) often increase to between 1.5 and 3.0 times normal within 2 to 3 weeks of beginning PN (215), although it should be recognized that these laboratory tests are both insensitive and nonspecific indicators of hepatic dysfunction and hepatic histopathology in this population (216). The elevation in AST and ALT levels is generally transient and may be related to a combination of cytokine release potentiated by the underlying illness and the absence of oral intake and portal nutrient absorption. Serum bilirubin concentration is rarely elevated in adults, although it may be observed in preterm infants. In these individuals, the incidence of PN-associated liver disease ranges between 15% and 85% across various centers, although in the largest study of 42 infants, Sondheimer et al. described an incidence of 67%, with 13% having progressed to hepatic failure after only 6 weeks of PN (217). In adults, the prevalence of significant liver enzyme abnormalities (defined as elevations in the levels of at least two of the three enzymes—ALT, AST, or alkaline phosphatase—to >1.5 times the upper limits of normal for >6 months) was 55% at 2 years, 64% at 4 years, and 72% at 6 years in a group of 90 (57 with histologic data)

patients (218). The percentage of patients who developed more complicated liver disease with one or more of six characteristics—serum bilirubin concentration more than 3.5 mg/dL for at least 1 month, ascites, hepatic encephalopathy, variceal hemorrhage, factor V concentration less than 50%, or portal fibrosis or cirrhosis on biopsy—was 26% at 2 years, 39% at 4 years, 50% at 6 years, and 53% at 8 years. Elevation of serum bilirubin concentration may be an ominous sign in adults; the time between onset of this observation and death was a median of 10.8 months in one study (219). All patients with a total serum bilirubin concentration more than 3.6 ± 1.2 mg/dL eventually died within 6 to 12 months.

Pathophysiology

Patients with the least residual functional intestine are at greatest risk for development of total parenteral nutrition (TPN)-associated liver disease and subsequent death from hepatic failure (220). This observation suggests that the more severe the malabsorption, the greater the risk for liver disease.

Nutrient absorption and metabolism differ significantly between orally ingested nutrients and those that are intravenously infused. After absorption of orally ingested nutrients into the portal circulation, nutrient remnants undergo hepatic first pass metabolism. Subsequently, metabolized products are circulated systemically through the right side of the heart and eventually to the kidneys where additional metabolism, reabsorption, or excretion may occur. Intravenously infused nutrients bypass the portal circulation. These are first transported to the heart and then subsequently to the liver through the hepatic artery rather than through the portal vein. A sulfur amino acid, methionine, is normally metabolized to cysteine and other metabolites through the hepatic trans-sulfuration pathway. Cysteine concentration increases after an oral and enteral methionine load (221). However, when methionine is infused intravenously, serum cysteine is nearly undetectable (221).

There are several other products of the trans-sulfuration pathway for which methionine is a substrate, including carnitine and choline (222,223,224,225). Although carnitine concentrations are decreased by approximately 50% from normal in patients who receive long-term TPN compared with a decrease to 10% of normal in case of true carnitine deficiency (222,223,224), carnitine supplementation does not reverse either steatosis or liver test abnormalities (225,226). Therefore, it is unlikely that carnitine deficiency is a cause for TPN-associated liver disease.

Plasma free choline concentration is below normal in more than 90% of patients who require TPN (227). Choline is required for the synthesis of VLDL, which transports triglycerides from the liver. During choline deficiency, hepatic triglyceride accumulates because there is insufficient VLDL to transport the triglyceride from the liver. A significant negative correlation exists between plasma free choline concentration and AST/ALT in patients who require long-term TPN (227). Choline supplementation ameliorates hepatic steatosis and leads to improvement in AST, ALT, and alkaline phosphatase levels in patients who require long-term TPN (228,229). Choline deficiency may be a necessary but insufficient cause for the progression of steatosis to steatohepatitis and more severe forms of TPN-associated liver disease. A second hit, such as lipid peroxidation, may be required (230,231). In a choline-deficient rodent model, Eastin et al. found that animals treated with endotoxin (lipopolysaccharide [LPS]) had significantly worse hepatic aminotransferase abnormalities, as well as steatohepatitis, when compared with choline-sufficient animals treated with LPS that exhibited minor biochemical abnormalities and no histologic changes (232). Methionine metabolism is impaired and its concentration in the blood increased because of what appears to be an ineffective hepatic trans-sulfuration pathway with regard to the metabolism of its intravenously infused form (84,221). Studies in rabbis have suggested that this buildup of methionine may have additional toxic effects on the liver (233).

Plasma free choline concentration is also decreased in infants who require TPN (234), and in addition to malabsorption and lack of choline in TPN, this may be related to an incompletely developed hepatic trans-sulfuration pathway (235,236). This may also result in taurine deficiency, although taurine-supplemented TPN was not shown to result in a decreased incidence of hepatic abnormalities (237,238,239). Oral taurine supplementation results in an increased taurine to glycine conjugated bile acid ratio (240), although this has not been demonstrated with intravenously infused taurine. Infants also have additional risk factors for the development of TPN-associated liver disease. These include an increased incidence of infections and antibiotic use, many surgical procedures, hypoxia, hypotension, and many blood transfusions (241,242).

Several nutrient toxicities have also been implicated in the development of TPN-associated liver disease. These including dextrose overfeeding (>50 kcal/kg per day), which leads to an increased portal insulin to glucagon ratio (243,244). The increased insulin concentration inhibits mitochondrial carnitine acyltransferase, which is the rate-limiting factor for fatty acid oxidation (245), and increases hepatic acetyl-coenzyme A concentration and induction of acetyl-coenzyme A carboxylase, which stimulates fatty acid synthesis (246,247).

Massive lipid emulsion infusion (>2.5 to 3.0 g/kg per day) has been associated with lipid overload

syndrome and hypoxia, thrombocytopenia related to disseminated intravascular coagulation, cholestasis, and death (248,249), although two retrospective reviews have suggested that hepatic aminotransferase abnormalities are more likely when the lipid emulsion infusion exceeds 1.0 g/kg per day (218,219). It has been hypothesized that the large concentration of plant sterols (phytosterols) contained in lipid emulsion results in decreased bile acid secretion and decreased secretory function in isolated rat hepatocyte couplets (250). However, it remains unclear whether the increased serum phytosterol concentration observed in patients on TPN who receive lipid emulsion is the cause of, rather than the result of, decreased hepatic phytosterol metabolism from other factors including those discussed in the preceding text. Despite the association of phytosterolemia and lipid emulsion dose with TPN-associated liver disease, reduction of the lipid emulsion infusion has not resulted in alleviation or resolution of TPN-associated hepatic derangements (251). There has been no randomized placebo-controlled trial to implicate a causal relationship between lipid emulsion infusion at conventional doses and hepatic abnormalities.

Several case reports have suggested a role for manganese toxicity in the development of TPN-associated liver disease (252,253,254). However, given that the excretion route for manganese is through the biliary tract, the likelihood is that manganese accumulates in the liver in patients with preexisting cholestasis. Aluminum contamination, once a problem in TPN solutions and a cause of cholestasis in animal models (255,256,257), has largely been eliminated; specific components of TPN such as calcium, potassium, phosphate, and sodium salts may still contain significant aluminum contamination. However, the overall contribution of aluminum contamination to the TPN solution is negligible (258,259). Copper toxicity resulting from TPN has not been described as a potential etiology for TPN-associated liver disease. However, given that the primary route of excretion for copper, like manganese, is through the biliary system, these trace metals should be withheld from the TPN solutions in patients with cholestasis.

Treatment of Total Parenteral Nutrition–Associated Liver Disease

Few therapies for TPN-associated liver disease are available. Carbohydrate and lipid overfeeding should be avoided. Intravenous choline currently remains an investigational therapy. Ursodeoxycholic acid has been described in a single case report and in a case series of nine patients in adults (260,261) and in a retrospective review and two open-labeled studies in neonates (262,263,264). Although cholestasis was not eliminated, one study reported a significant decrease in the serum bilirubin concentration in neonates who were given 15 to 45 mg/kg per day (262). In another study, the use of metronidazole was associated with improvements in response to hepatic derangements, but the patients studied also had active Crohn's disease and were overfed, two factors that may also lead to abnormal hepatic aminotransferases (265).

Intestinal transplantation is an option for patients with progressive and irreversible TPN-associated liver disease. It must be recognized, however, that in many patients exhibiting an elevation in hepatic aminotransferase levels the disease may not progress to the more severe stage. Currently, other than residual bowel length, there are no known predictors that are useful in estimating the likelihood of development of progressive fibrosis and eventual hepatic failure. Early stages of TPN-associated liver disease may be reversible with isolated intestinal transplantation as absorption improves (266). However, patients with cirrhosis or portal hypertension require combined liver/small intestine transplantation for survival. It is important that such patients be referred early to a transplantation center that is experienced not only in intestinal transplantation but also in the medical management of patients with intestinal failure. An argument could be made that all patients with intestinal failure should be seen in specialized centers regardless of their need for transplantation, given that mortality is lower in such centers (267). In the United States, 74% of all patients on the transplantation list awaiting the availability of a small intestine also require a liver (268). Furthermore, among patients in the waiting list who are in need of combined small intestine/liver transplantation, the mortality is 55% (269). Post-transplantation survival is also inferior in patients undergoing combined small intestine/liver transplantation when compared with those who receive an isolated small intestinal graft (Table 13.8) (270,271).

Nutritional Management of Patients with Liver Disease

Alcoholic Hepatitis

The use of standard amino acids, usually given with hypotonic dextrose through a peripheral vein, has been reported in several prospective randomized controlled trials (PRCTs) involving patients with alcoholic hepatitis (272). Nutritional therapy often improved the response of standard markers of nutritional status and liver chemistries but did not affect morbidity and mortality. The clinical efficacy of peripheral parenteral amino acids in alcoholic hepatitis has been evaluated by

several groups (273). Most studies found improved histology or liver biochemistries in patients who received parenteral amino acids, and one study claimed that survival was improved. Some studies evaluated the use of nutritional therapy in conjunction with corticosteroids in patients with alcoholic hepatitis. One PRCT compared patients randomized to peripheral parenteral nutrition (PPN) (50 g glucose and 35 g amino acids daily), oxandrolone, the combination of PPN and oxandrolone, or no treatment for 21 days. Child-Pugh score improved significantly in those who received PPN with oxandrolone, but mortality rates were similar in all groups (274,275). Mendenhall et al. studied the effect of the combination of oxandrolone with a branched-chain amino acid (BCAA)-supplemented formula in patients with moderate or severe alcoholic hepatitis (276). On an intention-to-treat basis, mortality rates were similar in both treated and control groups of patients with severe alcoholic hepatitis. A subgroup analysis that included only those subjects with markers suggesting either advanced liver disease or moderate malnutrition found that hepatic function was improved. In addition, a significant reduction in 1- and 6-month mortality rates was observed in subjects who received nutritional therapy. Enteral tube feeding was compared to prednisolone (40 mg/day) in a group of 71 patients (80% with cirrhosis) who developed alcoholic hepatitis. Short-term mortality (during treatment) was similar between the two groups but was greater in the steroid group at 6 weeks; this was primarily related to infections (277).

Table 13.8. Survival Data for Small Intestine and Liver/Small Intestine Transplantation

Transplantation type	Number of transplantations	Months post-transplantation	Survival Survival rate	95% confidence limits
Intestine alone	351	12	83.41	(79.10,87.72)
		36	64.67	(58.40,70.93)
		48	58.34	(51.41,65.27)
Liver/intestine	317	12	60.37	(54.73,66.01)
		36	49.46	(43.47,55.44)
		48	46.38	(40.29,52.47)
Multivisceral	253	12	69.39	(63.33,75.44)
		36	55.67	(48.56,62.77)
		48	54.65	(47.40,61.91)

Based on Organ Procurement and Transplantation Network (OPTN) data as of December 5, 2005. This work was supported in part by Health Resources and Services Administration contract 231-00-0115. The content is the responsibility of the authors alone and does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does it mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Alcoholic Cirrhosis

The results of several PRCTs evaluating oral and enteral tube feeding in patients with alcoholic cirrhosis demonstrate that aggressive feeding can be given safely and may have clinical benefits (278,279,280). Oral or enteral tube feedings resulted in the delivery of the desired nutrient level and often provided twice the protein and calories ingested ad lib by the control group. Feedings were usually well tolerated and no serious adverse events were described. Patients who received nutritional support had evidence of improvements in hepatic function (as assessed by hepatic function studies and liver biochemistries), response to hepatic encephalopathy, and Child-Pugh classification, and sometimes improved survival in patients fed with a BCAA-enriched formula.

Several PRCTs have found that enteral nutrition support in patients hospitalized for complications of cirrhosis was well tolerated and improved liver function, response to hepatic encephalopathy, and Child-Pugh score (278,279,281,282,283). In addition, one study found a trend toward decreased in-hospital mortality (3 to 4 weeks) in patients with cirrhosis and PEM who were given enteral tube feeding with a BCAA-enriched formula (mean intake = 2,115 kcal/day; mortality 12%) compared with an ad lib hospital diet (mean intake = 1,320 kcal; mortality 47%) (283). However, the difference between groups may have been exaggerated by the unusually high mortality rate observed in the control group.

Hepatic Encephalopathy

Most patients with cirrhosis can tolerate an increasing amount of standard protein without worsening encephalopathy. Protein restriction should not be considered routine; in fact, protein requirements are increased in patients with end-stage liver disease. Portosystemic encephalopathy can and should be aggressively treated using standard therapy before protein restriction is instituted. Ingestion of excessive dietary protein has been identified as the precipitating factor for the development of hepatic encephalopathy only in 7% to 9% of patients (283). Adults require 1.0 to 1.5 g/kg per day of protein on the basis of their dry body weight and children may require, depending on their age, 2.5

to 3.0 g/kg per day to avoid a catabolic state. Dietary protein content should be incrementally increased as encephalopathy alleviates in those patients for whom protein restriction is necessary until the tolerance point is established. Several studies have suggested amelioration in hepatic encephalopathy when vegetable protein–based diets are prescribed (284,285,286,287). The efficacy of vegetable protein–based diets in ameliorating hepatic encephalopathy is probably not related to their amino acid composition because this diet is similar in BCAA composition to meat protein–based diets but is more likely related to the fiber content and stool-bulking properties of such a diet. Fecal volume and weight increase with a vegetable protein diet (284). Although the methionine content of animal protein–based meals is substantially greater than that contained in vegetable protein–based meals, methionine concentration was not reduced in patients with cirrhosis who were fed a vegetable protein diet (284,287). This may be related to decreased methionine metabolism through the trans-sulfuration pathway. A diet based solely on vegetable protein, however, is unpalatable and, therefore, not practical. Vegetable protein should, however, be incorporated into the usual diet.

Lactulose is a synthetic disaccharide that consists of galactose and fructose, neither of which is hydrolyzed by the human enterocyte. Fecal nitrogen excretion is enhanced with lactulose (288). This appears to be related to the enhancement of nitrogen retention by excreted colonic flora (289). Similar effects were observed in patients with cirrhosis who were fed vegetable protein diets (290), as well as in patients who received soluble-fiber (pectin) supplementation (291). Soluble fiber is fermented by colonic bacteria to short-chain fatty acids, among other metabolic products.

The use of formulas with high concentrations of BCAAs (e.g., leucine, isoleucine, and valine) and small amounts of aromatic amino acids (AAAs) (e.g., phenylalanine, tyrosine, and tryptophan, Table 13.9) has been proposed in patients with hepatic encephalopathy on basis of the observation that the ratio of plasma BCAAs to AAAs is reduced in patients who have cirrhosis (292). According to the false neurotransmitter hypothesis, restoration of a normal BCAA to AAA ratio in the plasma would lead to decreased AAA transport across the blood–brain barrier and, subsequently, lower serotonin synthesis in the brain (293,294).

BCAA-enriched liquid formulas, which contain approximately 35% of total amino acids in the form of BCAAs, may be useful in a small percentage of patients with cirrhosis who are truly intolerant to increasing dietary protein. As much as 80 g of protein given as BCAA-enriched solutions has been tolerated in patients who could not tolerate 40 g of standard dietary protein (295). The large expense of BCAA-enriched solutions discourages their use in most settings. The clinical efficacy of parenteral BCAA-enriched TPN solutions in patients with acute hepatic encephalopathy has been evaluated in nine PRCTs. Five of these trials were reviewed using meta-analytic methodology to pool data across studies (296). Patients who received BCAA-enriched solutions demonstrated a statistically significant improvement in mental recovery from high-grade encephalopathy during short-term (7 to 14 days) nutritional therapy. Considerable heterogeneity in mortality rates between studies precluded meaningful aggregation of mortality data. Although the pooled analysis of trials suggest a beneficial effect of BCAA-enriched formulas as primary therapy in patients with acute hepatic encephalopathy, the studies have several shortcomings that limit enthusiasm for this relatively expensive therapy. The control groups usually received suboptimal, and possibly harmful, nutritional support consisting of high-dextrose solutions without amino acids, and there is considerable heterogeneity in study design. Only one study compared BCAA-enriched TPN with a standard amino acid TPN solution. None of the studies reported on complications associated with nutritional therapy and none evaluated whether short-term benefits of nutritional therapy led to a long-term reduction in complications. A more recent review of the same studies included in the meta-analysis by Naylor et al. albeit with some additional data; Gluud; and Ericksson and Conn concluded that BCAA use had no effect on mortality and that current evidence-based literature did not support the routine use of BCAAs (297,298,299). Data from a more recent 1-year double-blind randomized multicenter trial of 174 patients found that treatment with oral BCAA supplementation significantly reduced length of hospital stay, mortality, and anorexia, and improved the Child-Pugh score, although compliance was poor (300). A Cochrane database review of the use of BCAA in patients with chronic hepatic encephalopathy identified 11 randomized trials that included a total of 556 patients (301). A significant improvement was seen with BCAA use in terms of severity of hepatic encephalopathy, although this positive outcome was generally observed only in studies of poor methodological quality; mortality was unaffected. The concept that increasing the plasma BCAA to AAA ratio leads to decreased encephalopathy has been questioned by some investigators because brain uptake of BCAA in some patients with cirrhosis may be similar to that in healthy controls (302) and may be a better correlate of hepatic function than the degree of encephalopathy in others (303,304).

Nutritional Support of the Peritransplantation Patient

The indications for the provision of nutritional support in the patient with hepatic failure awaiting transplantation, as well as the transplant recipient after

transplantation, are the same as those of other perioperative patients. Hypoglycemia is a life-threatening complication of hepatic failure due to impaired gluconeogenesis and increased glucose uptake caused by hyperinsulinemia, and patients may require a continuous glucose or dextrose supply (minimum 150 to 200 g/day). Intervention should be provided rapidly in malnourished patients because moderately severe and severe malnutrition are risk factors for poor wound healing, wound dehiscence, infection, and death postoperatively (188,189,190,191,192,193,194,195,196) or in patients who are unable to meet their daily nutritional requirements. There is however a paucity of data to support the effect of perioperative enteral supplements, tube feeding, or parenteral nutrition on transplant outcome. Nasoenteric feeding is the simplest measure and can be safely accomplished even in patients with esophageal varices. (305), although there are risks for aspiration and sinusitis. Oral intake should be encouraged unless contraindicated, even with supplemental tube feeding. A nasoenteric tube can remain in place for

up to 4 to 6 weeks. Bolus or overnight feeding through a percutaneously inserted gastrostomy tube may also be undertaken. In patients with ascites, a large-volume paracentesis should be undertaken before percutaneously inserted gastrostomy tube placement (306,307).

Sodium restriction is necessary in patients with ascites or edema, although free water should not be restricted in the absence of severe hyponatremia (sodium <120 meq/L).

Table 13.9. Parenteral and Enteral Branched-Chain Amino Acid Formulas

A. PARENTERAL

	Increased essential amino acids				Increased Branch-Chain Amino Acids				
Amino acid	**Aminess 5.2%**	**Aminosyn RF 5.2%**	**Nephramine II 5.4%**	**RenAmin 6.5%**	**Aminosyn HBC 7%**	**Branchamin 4%**	**FreAmine HBC 6.9%**	**HepatAmine 8%**	**Neonatal Formula Trophamine 6%**
Essential amino acids (g/dL)									
Lysine	0.06	0.50	0.64	0.45	0.26	0	0.41	0.61	0.49
Tryptophan	0.18	0.16	0.20	0.16	0.08	0	0.09	0.07	0.12
Phenylalanine	0.82	0.73	0.88	0.49	0.23	—	0.32	0.10	0.29
Methionine	0.82	0.73	0.88	0.50	0.21	—	0.25	0.10	0.20
Threonine	0.38	0.33	0.40	0.38	0.27	—	0.20	0.45	0.25
Leucine	0.82	0.73	0.88	0.60	1.58	1.38	1.37	1.10	0.84
Isoleucine	0.52	0.46	0.56	0.50	0.79	1.38	0.76	0.90	0.49
Valine	0.60	0.53	0.64	0.82	0.79	1.24	0.88	0.84	0.47
Nonessential amino acids (g/dL)									
Histidine[a]	0.41	0.43	0.25	0.42	0.15	—	0.16	0.24	0.29
Glutamate	—	—	—	—	—	—	—	—	0
Proline	—	—	—	0.35	0.45	—	0.63	0.80	0.41
Aspartate	—	—	—	—	—	—	—	—	0
Serine	—	—	—	0.30	0.22	—	0.33	0.50	0.23
Arginine	—	0.60	—	0.63	0.51	—	0.58	—	0.73
Alanine	—	—	—	0.56	0.66	—	0.40	0.77	0.32
Glycine	—	—	—	0.30	0.66	—	0.33	0.90	0.22
Tyrosine	—	—	—	0.04	0.03	—	—	—	0.14
Cysteine	—	—	<0.02	—	—	—	<0.02	<0.02	<0.02
Taurine	—	—	—	—	—	—	—	—	0.015

Value given for a single concentrated solution available in each product line.
[a]Contains sodium bisulfite. Product mentions are not intended as endorsements.
Histidine is considered essential for patients with renal failure.

B. ENTERAL

Enteral formula	Manufacturer	Form	Kcal/mL	Protein source	Medium-chain triglycerides to long-chain triglycerides ratio	Osm (mosm/L)
NUTRIHEP	Nestle	Liquid	1.5	Amino acids, whey protein (50% branched-chain amino acids)	66:34	690

Hepatic Aid II	Hormel	Powder	1.2	Amino acids (46% branched-chain amino acids)	No medium-chain triglycerides	560

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After liver transplantation, the indications for parenteral nutrition include the inability to utilize the gastrointestinal tract for feeding because of postoperative ileus, enterocutaneous or biliary fistulas, and so on. In general, most liver transplantation patients can safely receive tube feedings within 18 to 24 hours of transplantation (308). Jejunostomy tubes for postoperative feeding can be placed safely during the transplantation procedure (309).

Annotated References

Als-Nielsen B, Koretz RL, Kjaergaard LL, et al. Branched-chain amino acids for hepatic encephalopathy. *Cochrane Database Syst Rev* 2003;(2):CD001939.

These investigators identified 11 randomized trials through the Cochrane Hepatobiliary Group Controlled Trials Register, pharmaceutical companies, manual searches of biographies and journals, contacts with authors of trials, and other online searches that included 556 patients. Overall, BCAA significantly increased the number of patients who had improvement in response to hepatic encephalopathy, although there was no evidence that the use of BCAA affected other morbidity or survival. In addition, the trials in which benefit was observed in hepatic encephalopathy were generally of poorer quality in which there were breakdowns in randomization or blinding of the investigator and/or research subject.

Buchman AL, Dubin M, Moukarzel A, et al. Choline deficiency: a cause of hepatic steatosis associated with parenteral nutrition that can be reversed with an intravenous choline chloride supplementation. *Hepatology* 1995;22:1399–1403.

These investigators demonstrated in a 15-patient placebo-controlled trial that intravenous choline supplementation for up to 24 weeks leads to the resolution of hepatic steatosis in patients who required parenteral nutrition. The resolution of hepatic steatosis was associated with decreases in serum ALT and alkaline phosphatase concentrations. This study suggests choline is an essential nutrient for patients who require parenteral nutrition.

Cavicchi M, Beau P, Crenn P, et al. Prevalence of liver disease and contributing factors in patients receiving home parenteral nutrition for permanent intestinal failure. *Ann Intern Med* 2000;132:525–532.

These investigators retrospectively evaluated 90 long-term home TPN patients who had received TPN for a median of 6 months (range 3 to 132 months). The prevalence of complicated liver disease, primarily indicative of portal hypertension, was 26% at 2 years and 50% at 6 years. After a median of 26 months, 17 patients showed evidence of hepatic fibrosis and 5 patients had developed cirrhosis after a median of 37 months. Twenty-two percent of all deaths were caused by liver failure in this home TPN group. Patients with the least residual intestine and those who received more than 1g/kg per day of lipid emulsion were at greatest risk for the development of chronic cholestasis.

Eastin CE, McClain CJ, Lee EY, et al. Choline deficiency augments an antibody to tumor necrosis factor-α attenuates endotoxin-induced hepatic injury. *Alcohol Clin Exp Res* 1997;21:1037–1041.

These investigators showed that a choline-deficient diet leads to the development of hepatic injury in a rodent model but that the administration of endotoxin in the presence of choline deficiency was necessary to provoke the development of steatohepatitis and hepatic necrosis; endotoxin injection alone resulted in increased serum TNF concentration but was not associated with hepatic abnormalities.

Horowitz JH, Rypins EB, Henderson JM, et al. Evidence for impairment of transsulfuration pathway in cirrhosis. *Gastroenterology* 1981;81:668–675.

These investigators administered an oral loading dose of methionine and then measured the plasma elimination half-life, as well as methionine metabolic products obtained through the hepatic trans-sulfuration pathway, in normal volunteers and in patients with cirrhosis. In the patients with cirrhosis the basal plasma methionine concentration was significantly elevated, clearance was delayed, and urinary sulfate elimination was impaired. Homocystine, cystathionine, homoserine, and cystine did not accumulate in plasma or urine, which suggested that the block in the hepatic trans-sulfuration pathway in cirrhosis is above the level of homocystine synthesis.

Madden AM, Morgan MY. Resting energy expenditure should be measured in patients with cirrhosis, not predicted. *Hepatology* 1999;30:655–664.

These investigators compared estimated resting energy expenditure from indirect calorimetric measurements with several formulas for energy expenditure, including the Harris-Benedict equation and others, in 100 patients with cirrhosis and 41 controls. They found that estimates of energy expenditure were generally elevated even in patients with stable cirrhosis compared to controls, although the formula both over- and underestimated REE by 460 to 424 kcal/day. Therefore, REE should be estimated by indirect calorimetry if available when determining nutritional requirements. The amount of fat=free mass was actually the strongest predictor of REE.

Petrides AS, Stanley T, Matthews DE, et al. Insulin resistance in cirrhosis: prolonged reduction of hyperinsulinemia normalizes insulin sensitivity. *Hepatology* 1998;28:141–149.

Using a euglycemic insulin-clamp technique, these investigators found that insulin-mediated glucose uptake was significantly increased, which was accounted for entirely by the impairment of nonoxidative glucose disposal in 8 patients with stable cirrhosis when compared with healthy volunteers. When nonoxidative glucose disposal was normalized during octreotide infusion over 96 hours, insulin-mediated glucose utilization also became normal. This suggests that chronic hyperinsulinemia is a cause for insulin resistance in cirrhosis.

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Chapter 14
Hepatic Fibrosis

Scott L. Friedman

Key Concepts

- Hepatic fibrosis is a reversible wound-healing response characterized by the accumulation of extracellular matrix (ECM) or "scar"; it follows chronic, but not self-limited, liver disease. The ECM components in fibrotic liver are similar, regardless of the underlying cause.
- Activation of hepatic stellate cells is the central event in hepatic fibrosis. These perisinusoidal cells and related myofibroblasts from intra- and extrahepatic origins orchestrate an array of changes including degradation of the normal ECM of liver, deposition of scar molecules, vascular and organ contraction, and release of cytokines.
- Not only is hepatic fibrosis reversible, but it is also increasingly clear that cirrhosis may be reversible as well. The exact stage at which fibrosis/cirrhosis becomes truly irreversible, and its biological determinants, are not known.
- Antifibrotic therapies have entered clinical trials. Emerging therapies will be targeted to those patients with reversible disease. The paradigm of stellate cell activation provides an important framework for defining therapeutic targets. Currently, the most significant impediment to drug development is the lack of robust noninvasive markers of fibrosis to accurately assess response to therapy.

Fibrosis is a reversible scarring response that occurs in almost all patients with chronic liver injury. Ultimately, hepatic fibrosis leads to cirrhosis, characterized by nodule formation and organ contraction. The causes of cirrhosis are multiple and include congenital, metabolic, inflammatory, and toxic liver diseases (Table 14.1).

It is essential to understand the molecular underpinnings of fibrosis because the fibrotic response underlies all the complications of end-stage liver disease, including portal hypertension, ascites, encephalopathy, synthetic dysfunction, and impaired metabolic capacity. Therefore, fibrosis is deleterious both by its direct effects on cellular function and by its mechanical contribution to increased portal resistance.

This chapter reviews the significant progress made toward elucidating the cellular basis of hepatic fibrosis and how these insights are leading to advances in the diagnosis and treatment of chronic liver disease.

The Biologic Basis of Hepatic Fibrosis

General principles

1. *Hepatic fibrosis is a wound-healing response* in which the damaged regions are encapsulated by extracellular matrix (ECM), or scar. In all circumstances, the composition of the hepatic scar is similar. The cells and soluble factors participating in this response in the liver are also similar to those involved in parenchymal injury to the kidney, lung, or skin. This understanding has helped to identify underlying mechanisms and will likely lead to new therapies for fibrotic diseases of many organs including the liver.
2. *Myofibroblast-like cells produce hepatic fibrosis regardless of the underlying cause*. As discussed in the subsequent text, the activated hepatic stellate cell is the

key cellular source of these myofibroblasts, but additional origins of fibrogenic cells may include peribiliary fibroblasts, bone marrow, and even transdifferentiation from epithelial cells. How stellate cells and related cells are activated in response to so large a variety of hepatic insults—from inborn metabolic defects to chronic viral hepatitis—is being increasingly revealed.

Table 14.1. Causes of Fibrosis and Cirrhosis

1. **Presinusoidal fibrosis** Schistosomiasis
   Idiopathic portal fibrosis
2. **Parenchymal fibrosis**
   A. Drugs and toxins Alcohol
      Methotrexate
      Isoniazid
      Vitamin A
      Amiodarone
      Perhexiline maleate
      α-Methyldopa
      Oxyphenisatin
   B. Infections
      Chronic hepatitis B, C
      Brucellosis
      Echinococcosis
      Congenital or tertiary syphilis
   C. Autoimmune disease
      Autoimmune hepatitis
   D. Vascular abnormalities
      Chronic passive congestion
      Hereditary hemorrhagic telangiectasia
   E. Metabolic/genetic diseases
      Wilson disease
      Genetic hemochromatosis

α_1-Antitrypsin deficiency
Carbohydrate metabolism disorders
Lipid metabolism disorders
Urea cycle defects
Porphyria
Amino acid metabolism disorders
Bile acid disorders

F. Biliary obstruction
Primary biliary cirrhosis
Secondary biliary cirrhosis
Cystic fibrosis
Biliary atresia/neonatal hepatitis
Congenital biliary cysts

G. Idiopathic/miscellaneous
Nonalcoholic steatohepatitis
Indian childhood cirrhosis
Granulomatous liver disease
Polycystic liver disease

3. **Postsinusoidal fibrosis**
Sinusoidal obstruction syndrome (veno-occlusive disease)

3. *Hepatic fibrosis follows chronic, not self-limited injury*. For example, patients surviving fulminant hepatitis do not develop scarring despite an abundance of fibrogenic stimuli, unless chronic injury follows. Moreover, even fibrosis associated with sustained injury is often reversible. The reason for fibrosis reversibility, even in chronic liver disease, is not certain, but may be related to the relative activity of matrix-degrading enzymes and their inhibitors, as well as the relative extent of collagen cross-linking.

4. *Fibrosis occurs earliest in regions in which injury is most severe*. This is especially true in chronic inflammatory liver disease due to alcohol or viral infection. For example, pericentral injury is a hallmark of alcoholic hepatitis, and the development of pericentral fibrosis (also known as sclerosing hyaline necrosis or perivenular fibrosis) is an early marker of likely progression to panlobular cirrhosis.

Extracellular Matrix Composition of the Normal Liver and Hepatic SCAR

ECM refers to the array of macromolecules comprising the scaffolding of normal and fibrotic liver. The components of hepatic ECM include several families of structural and supporting molecules: Collagens, noncollagen glycoproteins, matrix-bound growth factors, glycosaminoglycans, proteoglycans, and matricellular proteins. Of the 20 types of collagens characterized thus far, 10 have been identified in the liver. Remarkable progress has been made in identifying new members of these families and in understanding how these molecules interact in a defined, stoichiometric manner to affect cell behavior (see ref. 1 for review). Matrix molecules are also recognized in a variety of new roles, including their identification as transmembrane transducers of extracellular

signals.

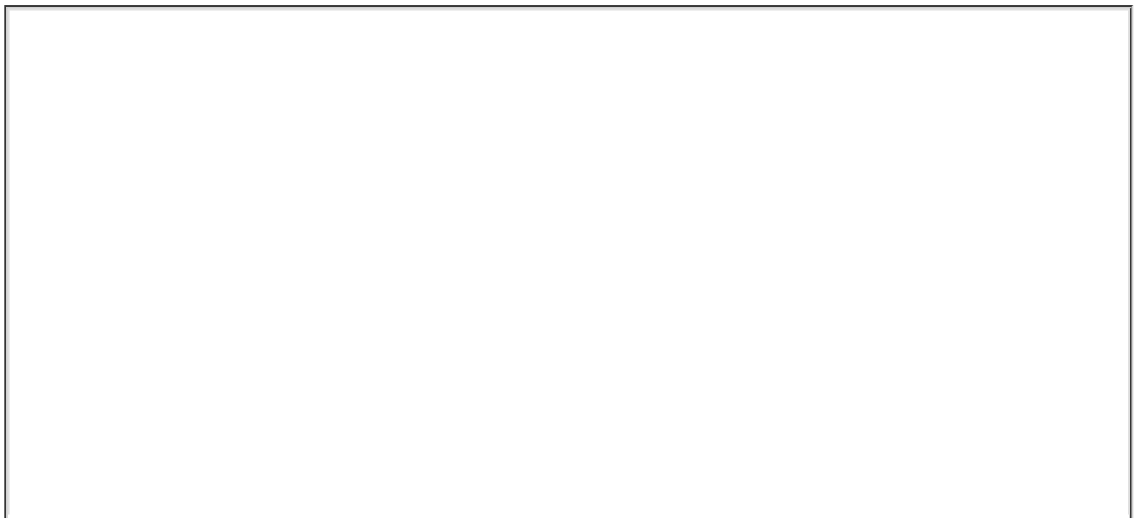
In the normal liver, so-called fibril-forming collagens (types I, III, V, and XI) are largely confined to the capsule, around large vessels, and in the portal triad, with only scattered fibrils containing types I and III collagen in the subendothelial space. Smaller amounts of other collagens including types VI, XIV, and XVIII can be found. Glycoproteins and matricellular proteins are also present, including subendothelial deposits of fibronectin, laminin, tenascin, secreted protein, acidic and rich in cysteine (SPARC), and von Willebrand's factor. Proteoglycans primarily consist of heparan sulfate proteoglycans including perlecan, as well as small amounts of decorin, biglycan, fibromodulin, aggrecan, glypican, syndecan, and lumican (1).

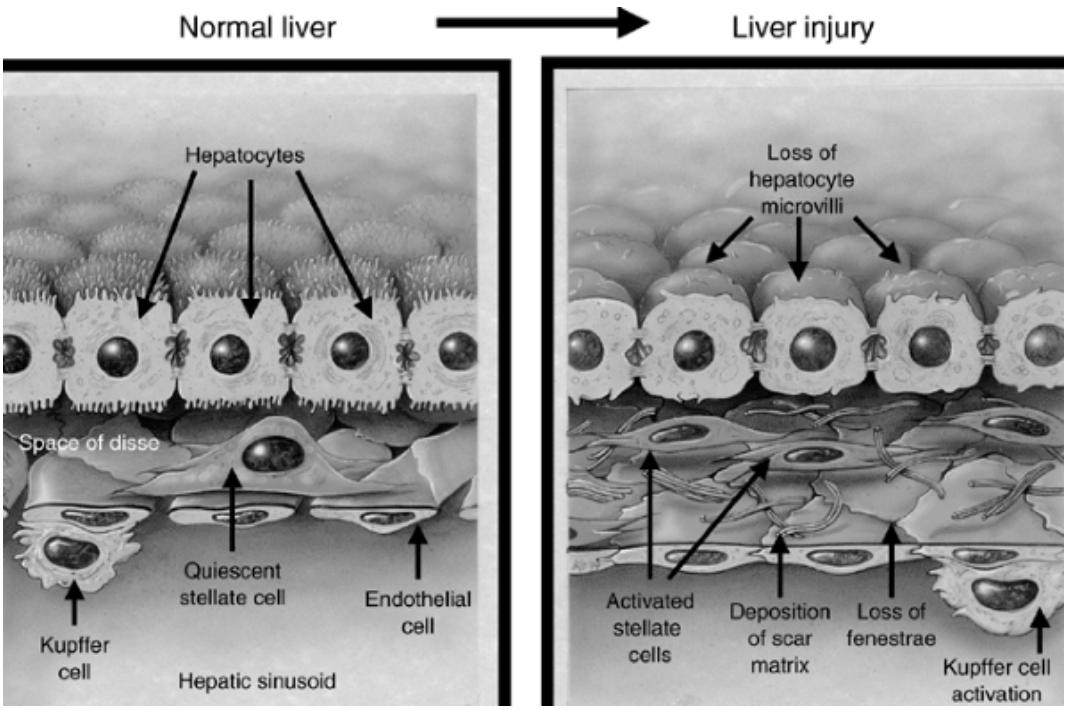
The fibrotic liver is characterized by both quantitative and qualitative differences in the matrix composition compared to the normal liver; as noted, these changes are similar regardless of the type of liver injury. Total collagen content increases 3- to 10-fold, although the collagen is not "abnormal" in sequence or structure. Overall, there is a marked increase in "interstitial matrix" that is typical of the healing wound, which includes fibril-forming collagens (types I, III, and V) and some non–fibril-forming collagens (types

IV, VI), several glycoproteins (e.g., cellular fibronectin, laminin, SPARC, osteonectin, tenascin, and von Willebrand's factor), a large number of proteoglycans and glycosaminoglycans (e.g., perlecan, decorin, aggrecan, lumican, fibromodulin), and related receptors, including dystroglycan (2).

Biologic Activity of Extracellular Matrix in Liver

ECM is a dynamic regulator of cell function and not an inert "ground substance." Early, subendothelial matrix accumulation leading to "capillarization" of the subendothelial space of Disse is a key event and may be more important than overall increases in matrix content (Fig. 14.1). The basement membrane constituents within the subendothelial space may be essential for preserving the differentiated functions of hepatocytes, hepatic stellate cells, and endothelial cells. Replacement of the normally low-density matrix by interstitial matrix directly perturbs hepatocyte function and could explain the synthetic and metabolic dysfunction that is characteristic of more advanced fibrosis and cirrhosis. This high-density matrix also activates stellate cells (3) and leads to a decrease in fenestrations of sinusoidal endothelial cells, which could impair the transport of solutes from the sinusoid to hepatocytes.





▪ **Figure 14.1** Matrix and cellular alterations in hepatic fibrosis. Changes in the subendothelial space of Disse as fibrosis develops in response to liver injury include alterations in both cellular responses and extracellular matrix composition. Stellate cell activation leads to an accumulation of scar (fibril-forming) matrix. This in turn contributes to the loss of hepatocyte microvilli and sinusoidal endothelial fenestrae, which results in the deterioration of hepatic function. Kupffer cell (macrophage) activation accompanies liver injury and contributes to the paracrine activation of stellate cells. (From Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247–2250, with permission.)

In liver injury, an increase in cellular fibronectin levels is among the first matrix alterations seen within this region. A consequence of this change is the activation of hepatic stellate cells and acceleration of fibrosis (See "Cellular Sources of Extracellular Matrix").

Altered cellular behavior induced by matrix alterations is typically mediated by cell membrane receptors. *Integrins*, a large family of homologous membrane linker proteins, are the best characterized type of ECM receptor. Integrins are noncovalent αβ heterodimers that consist of a large extracellular domain, a membrane-spanning domain, and a cytoplasmic tail (see refs 4,5,6 for reviews and references therein).

Integrins control many cellular functions including gene expression, growth, and differentiation. A growing number of α and β subunits have been identified, with each combination having a different cellular and ligand specificity. For many integrins, the tripeptide sequence Arg-Gly-Asp (RGD) present within the ligand is recognized by the receptor; this property underlies the experimental use of competitive RGD antagonists as therapies to block integrin function in a variety

of diseases including hepatic injury and fibrosis (See "Therapy of Hepatic Fibrosis").

Several integrin and nonintegrin receptors have been described in situ on hepatocytes and nonparenchymal cells (7,8,9,10,11,12). Upregulation of $\alpha_6\beta_1$ and $\alpha_2\beta_1$ receptors (10), both of which bind laminin, has been reported in experimental fibrosis. Studies have also defined the integrin phenotypes of the cell types isolated from the liver. In particular, stellate cells express integrin receptors for collagen and laminin (7,8,9,12), which may contribute to their activation and proliferation in response to the deposition of these matrix components during injury.

In addition to integrins, a growing number of other adhesion proteins and cell matrix receptors have been characterized, including cadherins and selectins, which mediate interactions between inflammatory cells and the endothelial wall (13). For example, upregulation of a tyrosine kinase receptor, discoidin domain receptor 2 (DDR2), has been identified during stellate cell activation, which signals in response to fibrillar collagens, leading to enhanced matrix metalloproteinase (MMP) expression and cell growth (14). The DDR family is the only family of receptor tyrosine kinases whose ligands are ECM molecules rather than peptide ligands, and their upregulation could be a critical requirement to perpetuate liver fibrosis.

ECM can also indirectly affect cell function through the release of soluble growth factors (cytokines), which is in turn controlled by local metalloproteinases. These include platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF), tumor necrosis factor α (TNF-α), basic fibroblast growth factor (bFGF), and vascular endothelial cell growth factor (VEGF) (1). Controlled release from ECM is a key mechanism for regulating cytokine activity because it provides a local, accessible source that is tightly regulatable through the actions of proteases and their inhibitors. In addition, ECM can regulate the activity of proteases through specific binding to collagens or fibronectins (1).

Cellular Sources of Extracellular Matrix in Normal and Fibrotic Liver

The identification of the cellular sources of ECM in hepatic fibrosis has been a significant advance that laid the groundwork for defining mechanisms of fibrosis and potential therapies. The *hepatic stellate cell* (previously called *lipocyte*, *Ito*, *fat-storing* or *perisinusoidal cell*) is the primary source of ECM in the normal and fibrotic liver. In addition, related mesenchymal cell types from a variety of sources may also make measurable contributions to total matrix accumulation, including classical portal fibroblasts (15,16,17) (especially in biliary fibrosis), bone marrow–derived cells (18), and fibroblast-derived epithelial–mesenchymal transition (EMT) (19). EMT is a well-characterized response of the kidney (19) to injury, but its role in liver injury has been less convincing.

Hepatic stellate cells are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells (see refs 20 and 21 for reviews). They are the primary sites for storing retinoids within the body. Stellate cells can be recognized by their vitamin A autofluorescence, perisinusoidal orientation, and expression of the cytoskeletal proteins desmin and glial acidic fibrillary protein. In strict terms, "stellate cells" may represent a

heterogeneous population of mesenchymal cells with respect to cytoskeletal phenotype, vitamin A content, and localization (22), but collectively they are the key fibrogenic cell type in the liver. Moreover, the remarkable plasticity of stellate cell phenotype has been documented in vivo and in culture, precluding a strict definition based only on the cytoskeletal phenotype (23,24). Stellate cells with fibrogenic potential are not confined to the liver and have been identified in the pancreas, for example, where they contribute to desmoplasia in chronic pancreatitis (25) and carcinoma (26).

Studies in situ in both animals and humans with progressive injury have defined a gradient of changes within stellate cells that are collectively termed *activation* (see subsequent text). Stellate cell activation refers to the transition from a quiescent vitamin A–rich cell to a highly fibrogenic cell characterized morphologically by the enlargement of rough endoplasmic reticulum, diminution of vitamin A droplets, ruffled nuclear membrane, appearance of contractile filaments, and proliferation. Cells with features of both quiescent and activated cells are often called *transitional cells*. As noted in the preceding text, proliferation of stellate cells occurs in regions of greatest injury, which is typically preceded by an influx of inflammatory cells and is associated with subsequent ECM accumulation.

Stellate cells have now been characterized in many human liver diseases. Alcoholic liver disease is the best studied example, with numerous reports documenting features of activation in situ (see ref. 27 for review); activation may occur even in the presence of steatosis alone without inflammation (28). Activated stellate cells have also been observed in viral hepatitis (29). In hepatocellular carcinoma, activated stellate cells contribute to the deposition of tumor stroma (30). Stellate cells have been characterized in a number of other human diseases including vascular disease, hematologic malignancy, biliary disease, mucopolysaccharidosis, acetaminophen overdose, leishmaniasis, allograft rejection, and in drug abusers.

Remarkably, very few studies have defined the cellular or matrix composition of congenital hepatic fibrosis, an entity whose pathogenesis is not clear. Current theories suggest that as in adults, congenital hepatic fibrosis

represents a final common pathway of fetal hepatic injury, whether from biliary malformations, viral infections (especially Cytomegalovirus), or other insults, with the stellate cell playing a significant role (31). Interestingly, the genetic knockout of a homeodomain transcription factor, Lhx2, leads to a congenital hepatic fibrosis–like picture (32), raising the intriguing but unproven suggestion that a similar genetic defect could underlie the human condition. Very few studies have examined specific mediators in this disease (33). It is unclear why fibrosis develops in weeks to months in utero, whereas it requires months to years in adults (See "Fibrosis Progression and Reversibility").

ECM production by sinusoidal endothelial cells, although less than that by stellate cells, is nonetheless an important component of early fibrosis. Like stellate cells, there is considerable heterogeneity of this cell type in normal and fibrotic livers. Endothelial cells from a normal liver produce types III and IV collagen laminin syndecans and fibronectin (34). After acute liver injury, increased expression of cellular isoforms of fibronectin by these cells is a key early event because their appearance creates a microenvironment that activates stellate cells.

Degradation of Extracellular Matrix

Degradation of extracellular matrix represents a key component of hepatic fibrosis for at least two reasons: (i) Early disruption of the normal hepatic matrix by matrix proteases ("pathologic" matrix degradation) hastens its replacement by scar matrix, which in turn has deleterious effects on cell function (See "Biologic Activity of Extracellular Matrix") and (ii) in established fibrosis in patients with chronic liver disease, there is an urgent need to resorb the excess wound matrix ("therapeutic" matrix degradation) in hopes of arresting or reversing hepatic dysfunction and portal hypertension. Because fibrosis reflects a balance between matrix production and degradation, this balance must be shifted in favor of degradation for any antifibrotic therapy to succeed.

There has been significant progress in elucidating the fundamental mechanisms of matrix remodeling and how these apply to hepatic fibrosis. An enlarging family of MMPs (also known as matrixins) has been identified, which are calcium-dependent enzymes that specifically degrade collagens and noncollagenous substrates (see refs 35 and 36 for review). Broadly, these fall into five categories based on substrate specificity: Interstitial collagenases (MMP-1, 8, 13) (37), gelatinases (MMP-2 and 9) and fibroblast activation protein (38), stromelysins (MMP-3, 7, 10, 11), membrane-type MMPs (MMP-14, 15, 16, 17, 24, 25), and a metalloelastase (MMP-12). Metalloproteinases are regulated at many levels to restrict their activity to discrete regions within the pericellular milieu. Inactive metalloproteinases can be activated through proteolytic cleavage by either membrane-type 1 matrix metalloproteinase (MT1-MMP) or plasmin and inhibited by binding to specific inhibitors known as *tissue inhibitor of metalloproteinases* (*TIMPs*). The stoichiometry and molecular basis for these interactions has been greatly clarified. For example, MT1-MMP and TIMP-2 form a ternary complex with MMP-2, possibly including $\alpha v\beta_3$ integrin, which is essential for optimal MMP-2 activity (36). Plasmin activity is controlled by its activating enzyme, uroplasminogen activator, and a specific inhibitor, plasminogen activator inhibitor 1, and can be stimulated by active transforming growth factor β_1 (TGF-β_1). Therefore, net collagenase activity reflects the relative amounts of activated metalloproteinases and their inhibitors, especially TIMPs. In addition to TIMPs, other protease inhibitors may affect the net degradative activity, including α_2-macroglobulin.

In the liver, "pathologic" matrix degradation refers to the early disruption of the normal subendothelial matrix, which occurs through the actions of at least four enzymes: *MMP*-2 (also called *gelatinase A* or *72-kDa type IV collagenase*) and *MMP-9* (*gelatinase B* or *92-kDa type IV collagenase*), which degrade type IV collagen; *membrane-type MMP-1 or 2*, which activates latent MMP-2; and *stromelysin-1*, which degrades proteoglycans and glycoproteins and also activates latent collagenases. Stellate cells are a key source of MMP-2 (34), MMP-13 in rodents (37), and stromelysin (34). Activation of latent MMP-2 may require interaction with hepatocytes (39,40). Markedly increased expression of MMP-2 is characteristic of cirrhosis (34,37). MMP-9 is locally secreted by Kupffer cells (36). Remarkably, Kupffer cells may exert a dual role in matrix degradation that differs between fibrosis progression and regression (41). Disruption of the normal liver matrix is also a requirement for tumor invasion and desmoplasia (42).

Failure to degrade the increased interstitial or scar matrix is a major determinant of progressive fibrosis. *MMP-1* is the main protease that can degrade type I collagen, the principal collagen in the fibrotic liver. However, the sources of this

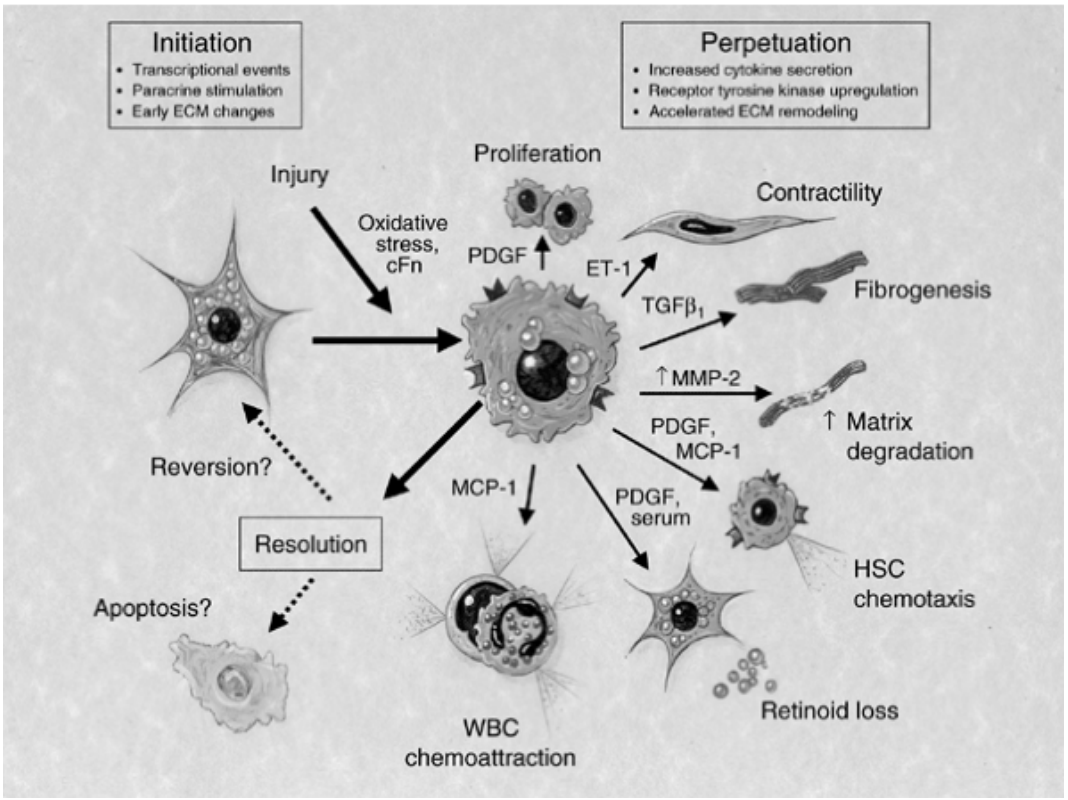
enzyme are not as clearly established as for the type IV collagenases. Stellate cells express MMP-1 messenger ribonucleic acid (mRNA), but little enzyme can be detected (43). More importantly, progressive fibrosis is associated with marked increases in TIMP-1 (44,45) and TIMP-2 (46), leading to a net decrease in protease activity and, therefore, more unopposed matrix accumulation. Stellate cells are the major source of these inhibitors (35). Sustained TIMP-1 expression is emerging as a key reason for progressive fibrosis, and its diminution is an important prerequisite to allow for reversal of fibrosis (see

P.400

subsequent text). Unique mechanisms of TIMP-1 regulation in stellate cells (47) offer the potential for selective inhibition of TIMP-1 expression to accelerate the resorption of scar matrix in patients with liver disease.

Stellate Cell Activation, The Central Event in Hepatic Fibrosis

It is useful to frame the pathophysiology of hepatic fibrosis around the mechanisms of hepatic stellate cell activation because this cell type is the major source of ECM. Activation consists of two major phases, *initiation* (also called a *preinflammatory stage*) and *perpetuation* (20) (Fig. 14.2). Initiation refers to early changes in gene expression and phenotype, which render the cells responsive to other cytokines and stimuli, while perpetuation results from the effects of these stimuli on maintaining the activated phenotype and generating fibrosis. Initiation is largely due to paracrine stimulation, whereas perpetuation involves autocrine and paracrine loops.



▪ **Figure 14.2** Phenotypic features of hepatic stellate cell activation during liver injury and resolution. Following liver injury, hepatic stellate cells

undergo "activation," during which they are transformed from quiescent vitamin A–rich cells into proliferative, fibrogenic, and contractile myofibroblasts. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, retinoid loss, and white blood cell (WBC) chemoattraction. Key mediators underlying these effects are shown. The fate of activated stellate cells during the resolution of liver injury is uncertain but may include reversion to a quiescent phenotype or selective clearance by apoptosis. ECM, extracellular matrix; cFn, cellular fibronectin; PDGF, platelet-derived growth factor; ET-1, endothelin-1; TGF-β_1, transforming growth factor β_1; MMP-2, matrix metalloproteinase-2; MCP-1, monocyte chemoattractant protein-1; HSC, hepatic stellate cell. (From Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247–2250, with permission.)

Initiation of stellate cell activation

The earliest changes in stellate cells are likely to result from paracrine stimulation by all neighboring cell types, including sinusoidal endothelium, Kupffer cells, hepatocytes, and platelets. As noted in the preceding text, early injury to endothelial cells stimulates the production of cellular fibronectin, which has an activating effect on stellate cells (48). Endothelial cells are also likely to participate in the conversion of TGF-β from the latent to active, profibrogenic form. Sinusoidal endothelial cells, normally fenestrated to allow rapid bidirectional transport of solutes between sinusoidal blood and parenchymal cells, may rapidly lose their fenestrations on injury and express proinflammatory molecules including intercellular adhesion molecule-1, VEGF, and adhesion molecules (42,49). Together with stellate cells, they activate angiogenic pathways in response to hypoxia associated with local injury or malignancy (42,50,51,52).

Hepatic inflammation, Kupffer cell infiltration, and activation also play prominent roles (53,54). Influx of

Kupffer cells coincides with the appearance of stellate cell activation markers. Kupffer cells can stimulate matrix synthesis, cell proliferation, and release of retinoids by stellate cells through the actions of cytokines (especially TGF-β_1) and reactive oxygen intermediates/lipid peroxides. Hepatic macrophages, most of which are likely to represent Kupffer cells, also have a complex role in matrix degradation in the liver (55).

Early stimulation of stellate cells by lipid peroxides in vivo may be important in many forms of liver fibrosis, particularly hepatitis C, nonalcoholic steatohepatitis (NASH), and iron overload (56). In fact, there is an increased prevalence of heterozygosity for mutation of the hemochromatosis gene in patients with NASH, suggesting a potentially synergistic relationship between fat and iron overload (57). Additionally, fibrosis is more likely among patients with NASH who are obese, which correlates with increased hepatic steatosis (58). Because antioxidant levels are typically depleted in cirrhotic liver as fibrosis advances, their loss could further amplify the injurious effects of lipid peroxides.

Hepatocytes, as the most abundant cells in the liver, are a potent source of these

fibrogenic lipid peroxides. There is an in situ correlation between the presence of aldehyde adducts and collagen gene expression by stellate cells (59), and peroxides stimulate collagen synthesis by cultured stellate cells (60). Steatosis in NASH and hepatitis C virus (HCV) correlates with increased stellate cell activation and fibrogenesis (61), possibly because fat represents an enhanced source of lipid peroxides. In culture, the activation of stellate cells is provoked by the generation of free radicals and is blocked by antioxidants (62).

Whereas hepatocyte necrosis associated with lipid peroxidation is considered a classical inflammatory and fibrogenic stimulus, recent findings also implicate apoptosis, or programmed cell death, in the fibrogenic response. Apoptotic fragments released from hepatocytes are fibrogenic toward cultured stellate cells (63) and activate Kupffer cells (64). Also, Fas-mediated hepatocyte apoptosis in vivo in experimental animals is fibrogenic (65).

Platelets are often overlooked as a paracrine stimulus, but, in fact, they are a potent source of growth factors and are present within the injured liver (66). Potentially important platelet mediators include PDGF, TGF-β_1, and epidermal growth factor (EGF).

Perpetuation of stellate cell activation

Perpetuation of stellate cell activation involves at least seven discrete changes in cell behavior: (a) Proliferation, (b) chemotaxis, (c) fibrogenesis, (d) contractility, (e) matrix degradation, (f) retinoid loss, and (g) white blood cell (WBC) chemoattractant and cytokine release. Either directly or indirectly, the net effect of these changes is to increase the accumulation of ECM. For example, proliferation and chemotaxis lead to increased numbers of collagen-producing cells, but there is also more matrix production per cell. Cytokine release by stellate cells can amplify the inflammatory and fibrogenic tissue responses, and matrix proteases may hasten the replacement of normal matrix with a matrix typical of the wound "scar."

1. *Proliferation*. PDGF is the most potent stellate cell mitogen identified (67). Induction of PDGF receptors early in stellate cell activation increases responsiveness to this potent mitogen (68). Downstream pathways of PDGF signaling have been carefully characterized in stellate cells (69). In addition to proliferation, PDGF stimulates Na^+/H^+ exchange, providing a potential site for therapeutic intervention by blocking ion transport (See "Therapy of Hepatic Fibrosis") (70). Transgenic expression of PDGF-C in mice leads to both hepatic fibrosis and carcinoma (71). Other compounds with mitogenic activity in stellate cells and a potential role in fibrogenesis include VEGF (51), thrombin and its receptor (72,73), EGF, TGF-α, keratinocyte growth factor (74), and bFGF (75). Signaling pathways for these and other mitogens have been greatly clarified in stellate cells, offering many potential sites for therapeutic intervention (see ref. 69).
2. *Chemotaxis*. Stellate cells can migrate toward cytokine chemoattractants (50,69) mediated by a number of transmembrane receptors (69,76,77), which is characteristic of wound-infiltrating mesenchymal cells in other tissues as well. Chemotaxis of stellate cells explains in part why stellate cells align within inflammatory septae in vivo.
3. *Fibrogenesis*. Increased matrix production is the most direct way by which stellate cell activation generates hepatic fibrosis. Among the components of

the hepatic scar, collagen type I is the best studied, and numerous reports describe the regulation of the collagen I gene in stellate cells.

The most potent stimulus for collagen I production is TGF-β_1, which is derived from both paracrine and autocrine sources; TGF-β_1 also stimulates the production of other matrix components including cellular fibronectin and proteoglycans (78). TGF-β_1 stimulates collagen in stellate cells through a hydrogen peroxide–, p38 mitogen-activated protein kinase (MAPK)-, and CCAAT/enhancer binding protein-β (C/EBPβ)-dependent mechanism (79,80). TGF-β expression is increased in patients with chronic hepatitis C, emphasizing the potential importance of this cytokine in chronic liver disease (81). Similarly, serum levels of TGF-β correlate with the risk of veno-occlusive disease after bone marrow transplantation (82). Also, stellate cell responsiveness to TGF-β_1 is increased during activation by enhanced ligand binding to its cognate receptors (78). Signaling molecules downstream

of TGF-β include a family of bifunctional molecules known as *Smads*, on which many extracellular and intracellular signals converge to fine-tune and additionally enhance the effects of TGF-β downstream of its receptors during fibrogenesis (83,84). The response of Smads in stellate cells differs between acute and chronic injury to further favor matrix production (85). Smads 2 and 3 elicit distinct signaling responses that favor stellate cell activation and fibrogenesis (69), whereas Smad 7 is inhibitory, making it an attractive molecule to utilize in antifibrotic therapies (86) (see subsequent text).

Continued understanding of collagen gene transcription, translation, and mRNA stability have emphasized the many sites of regulation that may affect collagen production (87,88,89,90).

Lipid peroxidation products are emerging as important stimuli to ECM production (91), particularly when derived from hepatocytes (62). Their effects may be amplified by the loss of the antioxidant capacity of stellate cells as they are activated (92). These important insights have provoked efforts to use antioxidants as therapy for hepatic fibrosis (See "Therapy of Hepatic Fibrosis"). Stimulation of fibrogenesis may be especially critical to the pathogenesis of NASH, wherein fat provides a large reservoir of potential reactive oxygen species (93,94,95). In addition to TGF-β_1, CTGF (now referred to as CCN2) is also a potent TGF-β-regulated fibrogenic cytokine (96,97).

4. *Contractility*. Contractility of stellate cells may be a major determinant of early and late increases in portal resistance during liver fibrosis. Activated stellate cells impede portal blood flow by both constricting individual sinusoids and contracting the cirrhotic liver because the collagenous bands typical of end-stage cirrhosis contain large numbers of activated stellate cells (see ref. 98 for review).

   As stellate cells become contractile, they have increased expression of the cytoskeletal protein, α-smooth muscle actin. If smooth muscle actin is required for contraction, then inactivating it could represent a therapeutic target for treating portal hypertension.

   The major contractile stimulus for stellate cells is endothelin-1, whose receptors are expressed on both quiescent and activated stellate cells but whose subunit composition may vary (98). On activation, the receptor

expression does not increase, unlike that of PDGF receptors, but there is a shift in the type of endothelin receptor that predominates, combined with increased sensitivity to autocrine endothelin-1 (99,100). Increased endothelin levels result from increased endothelin-converting enzyme (ECE) activity because of stabilization of the ECE mRNA (101). Contractility of stellate cells in response to endothelin-1 has also been documented in vivo (102).

Another key contractile mediator in activated stellate cells is angiotensin II, which is synthesized by activated stellate cells in a nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH)-dependent pathway (103,104,105). These findings are particularly relevant to human disease because antagonism of this pathway is an attractive antifibrotic therapy using a variety of safe, well-tolerated medications that are already available (106) (See "Therapy of Hepatic Fibrosis"). Contraction may also be induced in activated stellate cells by vasopressin (107), substance P (108), and atrial natriuretic peptide (109).

Locally produced vasodilator substances may counteract the constrictive effects of endothelin-1 (110,111). Nitric oxide, which is also produced by stellate cells, is a well-characterized endogenous antagonist to endothelin. During acute endotoxemia, stellate cell production of nitric oxide is increased. In vivo studies suggest that carbon monoxide also mediates sinusoidal relaxation through its effects on stellate cells (100,112).

5. *Matrix degradation*. Quantitative and qualitative changes in matrix protease activity play an important role in the ECM remodeling that accompanies fibrosing liver injury (see "Extracellular Matrix Degradation"). Stellate cells express virtually all the key components required for pathologic matrix degradation and, therefore, play a key role not only in matrix production but also in matrix degradation.

6. *Retinoid loss and nuclear receptor signaling*. As stellate cells are activated, they lose their characteristic perinuclear retinoid (vitamin A) droplets and acquire a more fibroblastic appearance. In culture, retinoid is stored as retinyl esters, whereas as stellate cells are activated, the retinoids are released outside the cell as retinol, suggesting that there is intracellular hydrolysis of esters before export (113). However, it is unknown whether retinoid loss is required for stellate cells to be activated, and which retinoids might accelerate or prevent activation in vivo. For example, one retinoic acid analog, 9-*cis*-retinoic acid, stimulates hepatic fibrosis in rats by increasing the activation of latent TGF-β_1 (114).

   Several nuclear retinoid receptors have been identified in stellate cells (115,116,117,118), molecules that bind intracellular retinoid ligands and regulate gene expression, but it is uncertain whether they play a regulatory role in fibrogenesis. The question has important clinical implications as efforts to use retinoids therapeutically are being developed (See "Therapy of Hepatic Fibrosis"). Recently, peroxisome proliferator-activated receptors (PPARs), in particular PPARγ, have been identified in stellate cells, and their expression increases with activation (116). Ligands for this newly identified nuclear receptor family downregulate stellate cell activation (116,119). Similarly, farnesoid X receptor (FXR) not only regulates a range of genes regulating choleresis but, in stellate cells, may also drive antifibrotic pathways alone or by converging with PPARγ signaling (115,120,121).

In contrast, PPARβ ligands stimulate stellate cell proliferation (117).

7. *Inflammatory signaling and WBC chemoattraction*. Stellate cells are assuming an increasingly central role in our understanding of hepatic inflammation. They can amplify the inflammatory response by inducing infiltration of mono- and polymorphonuclear leukocytes. Activated stellate cells produce chemokines that include monocyte chemoattractant protein-1 (110), CCL21 (29), regulated on activation, normal T cell expressed and secreted (RANTES), and CCR5 (122). They also express toll-like receptors (123), indicating a capacity to interact with bacterial lipopolysaccharide, which in turn stimulate stellate cells (124). Stellate cells can also function as antigen-presenting cells (125) that can stimulate lymphocyte proliferation or apoptosis (126). In addition to this mononuclear cell chemoattractant, stellate cells produce neutrophil chemoattractants, which could contribute to the neutrophil accumulation characteristic of alcoholic liver disease.

In addition to regulating leukocyte behavior, stellate cells may also be affected by specific lymphocyte populations. For example, CD8 cells harbor more fibrogenic activity toward stellate cells than CD4 cells (127), which may explain in part the increased hepatic fibrosis seen in patients with HCV/human immunodeficiency virus (HIV) coinfection, in which CD4/CD8 ratios are reduced, compared to that in patients monoinfected with HCV alone.

Transcriptional regulation of stellate cell activation

There have been many advances in dissecting pathways of membrane and intracellular signaling and transcriptional gene regulation in activated hepatic stellate cells that are too numerous to detail here (128). As noted in the preceding text, the growing number of transcription factors and signaling molecules may regulate stellate cell behavior, including PPARα, β and γ (117,129), cysteine- and glycine-rich protein 2 (CRP-2) (130), retinoid receptors (131), nuclear factor-κB (NF-κB) (123), Jun D (128), Krüppel-like factor 6 (132), Foxf1 (133), Lhx2 (32), and MEF2 (134), among others (128,129).

Disease-Specific Mechanisms Regulating Hepatic Fibrosis—Hepatitis C Virus and Nonalcoholic Steatohepatitis

In addition to generic mechanisms of fibrogenesis common to all experimental and human liver disease, there has been progress in elucidating disease-specific mechanisms, in particular in HCV and NASH. A recent study has raised the possibility that stellate cells might be infected by HCV by identifying the expression of putative HCV receptors in activated stellate cells, including CD80, low-density lipoprotein receptor, and C1q (135). Moreover, adenoviral transduction of HCV nonstructural and core proteins induces stellate cell proliferation and release of inflammatory signals (135). The E2 protein of HCV can interact directly with CD81, a stellate cell plasma membrane receptor (136). Moreover, hepatocytes in culture that support HCV replication generate paracrine factors that also stimulate stellate cells (137). In HCV-infected liver, chemokines and their receptors are upregulated, stimulating lymphocyte recruitment (29). HCV proteins may also interact directly with sinusoidal endothelium (138). A ductular reaction with portal expansion also correlates with fibrosis progression

(139). Steatosis and apoptosis in HCV may accelerate fibrosis (140), even in patients without NASH, and steatosis has emerged as an important determinant of fibrosis progression in HCV (141).

The rising prevalence of obesity in the United States and Western Europe is associated with an alarming increase in NASH (58) leading to advanced fibrosis and cirrhosis and accelerated mortality (142). Leptin, a circulating adipogenic hormone that is proportionate to the adipose mass in circulating blood, has been clearly related to stellate cell fibrogenesis (143,144,145) and requires sympathetic neurotransmission (146). Sources of fibrogenesis are likely to be both endocrine and autocrine, associated with enhanced signaling through the leptin receptor, which is upregulated during stellate cell activation (143,147). Concurrently, downregulation of adiponectin in obesity, a counter-regulatory hormone, may amplify the fibrogenic activity of leptin. This possibility is supported by findings in mice lacking adiponectin, which have enhanced fibrosis after toxic liver injury (148).

Clinical Aspects of Hepatic Fibrosis

Fibrosis Progression and Reversibility

The rate of progression of fibrosis in an individual patient with chronic liver disease cannot be predicted with certainty. However, some general rules apply:

1. *Fibrosis usually requires at least several months to years of ongoing insult*. Three exceptions in adults are sinusoidal obstruction syndrome (SOS) (previously referred to as veno-occlusive disease), subfulminant drug-induced liver injury, and mechanical biliary obstruction, in which fibrosis can progress more rapidly. In SOS, a dramatic deposition of ECM in pericentral zones associated with activated stellate cells can

follow within weeks of myeloablative therapy, leading to rapid onset of ascites and possibly death. In infants with neonatal obstruction, fibrosis can also develop in utero or within weeks of birth. Mechanisms underlying these examples of "fulminant fibrosis" remain obscure, but stellate cell activation and upregulation of fibrogenic cytokines accompany these fibrotic states as they do in more common forms of hepatic fibrosis.

In contrast to these relatively rare forms of "fulminant" fibrosis, in patients with HCV infection, the median time to overall progression has been estimated at 30 years (149), ranging from 13 to 42 years, depending on the presence of other risk factors. Risk factors for more rapid progression include alcohol abuse, older age at the time of infection, steatosis (as reflected by elevated body mass index), immunosuppression, and male gender. Importantly, the progression does not correlate with viral load or genotype, strongly implicating host response and genotype as determinants of fibrosis progression. In fact, there is increasing appreciation of host genetic determinants that influence fibrosis (150), and large-scale efforts are under way within the commercial sector to define "gene risk signatures" through whole-genome analysis of single nucleotide polymorphisms in patients with HCV and nonalcoholic fatty liver disease (151). Similar studies in animals have successfully identified "fibrogenic loci" whose gene products may modulate the inherent fibrotic response (152).

2. *The severity of inflammation, necrosis, and injury usually correlate with the rate of progression*, as documented in studies of alcoholic liver disease and hepatitis C (153,154,155). Indeed, patients with persistently normal alanine aminotransferase and HCV are less likely to progress (156), indicating decreased overall necroinflammation during the course of their disease.

3. *Concurrent hepatic insult by more than one agent is synergistic for the progression of fibrosis*. This has been especially well documented in patients with HCV infection who abuse large amounts of alcohol but is also true for those with viral coinfection, including hepatitis B virus (HBV) with either HCV or hepatitis delta virus (HDV) (157), as well as HCV with HIV (149,158).

   Iron overload, whether primary or secondary, is a risk factor for fibrosis progression (94). In genetic hemochromatosis and rodent models of iron overload, a threshold iron concentration of 22,000 μg/g of dry weight has been identified. Higher iron content also correlates with increased inflammation and fibrosis in HCV (159) and synergizes inflammation. Increased steatosis is also associated with increased fibrosis in patients infected with HCV (160) or in those who have NASH (161). Other risk factors for fibrosis in patients with NASH include older age, obesity, and presence of diabetes mellitus (162,163,164).

4. *The exact moment when fibrosis becomes irreversible is not known*, in terms of either a histologic marker or a specific change in the matrix composition or content. Dense cirrhosis, with nodule formation and portal hypertension, is generally considered irreversible, but more intermediate lesions can show remarkable reversibility. It is important to consider the possibility that even advanced stages of fibrosis/cirrhosis might be reversible as clinical trials of antifibrotics emerge. Moreover, just as fibrosis may progress over decades, the reversal of fibrosis may also require many years, a fact that will influence the design of antifibrotic trials. Irreversibility may be conferred by the density and acellularity of the septal scars, leading to the loss of sources of interstitial collagenases.

   Reversibility of cirrhosis has now been well documented in a number of diseases including HCV, HBV, HDV, and alcoholic liver disease (165,166,167,168). In mechanical biliary obstruction due to chronic pancreatic disease, surgical decompression can also lead to the reversal of fibrosis (169), a finding supported in animal models (170).

   Animal studies have also yielded significant insight into the mechanisms of fibrosis regression. Sustained experimental liver injury by carbon tetrachloride (CCl_4) leads to delayed reversibility because of thick collagen bands, high levels of TIMP-1, and significant collagen cross-linking by tissue transglutaminase (171), whose expression is increased in activated stellate cells (172). A major role of TIMP-1 is to inhibit the apoptosis of activated stellate cells, thereby allowing these fibrogenic cells to persist in the injured liver (173), with sustained NF-κB signaling providing an intracellular molecular signal to preserve this activated state (174,175). Mice that overexpress TIMP-1 in the liver indeed have a delayed regression of experimental fibrosis (176), reinforcing its role in preventing reversibility. In addition, extracellular survival signals, some of which are bound to surrounding ECM, may further prevent apoptosis.

5. *Host genotype is an intrinsic determinant of fibrosis progression*. In addition

to rapid advances in identifying host genes that regulate the risk of fibrosis progression, characterization of the host immune factors contributing to fibrosis could represent an important insight into the pathogenesis of fibrosis in humans. In HCV infection, the predominance of a Th1 cytokine response has been correlated with more severe injury and fibrosis (177,178). Studies in mice suggest that the immune phenotype is a key determinant of fibrosis in schistosomiasis (179) and toxic liver injury (180). Fibrosis is also accelerated in patients with HCV who are coinfected with HIV (158), further implicating a role of the immune system in modulating fibrosis. In contrast, estrogen in women may provide some protection against fibrosis (181) because fibrosis progression is slower in women than in men (182).

Diagnosis and Assessment of Hepatic Fibrosis

Accurate assessment of the extent of fibrosis is essential to guide management and predict prognosis in patients with chronic liver injury. Histologic assessment of a liver biopsy specimen remains the "gold standard" for quantifying fibrosis, with increasing interest in the use of noninvasive markers to allow more frequent sampling and avoid the risks of percutaneous biopsy.

Histologic and morphometric methods

Three methods of assessment of histologic fibrosis are in widest use—the Ishak score (183), the Metavir score (184), and the Desmet/Scheuer staging system (185). Each relies on progressive development of periportal, then septal fibrosis, and finally nodule formation. The key distinguishing feature is the presence of two cirrhotic stages (5 and 6) in Ishak, and only one (F4) in the Metavir system. Interobserver variation is low in both systems, especially if pathologists have been "trained" before the use of these systems. However, sampling error may exceed 30%, as demonstrated by a study in which laparoscopic biopsies were obtained from both lobes of patients with HCV, yet 33% of the 124 patients had differences of at least one stage between the two lobes (186). Two key features determining the accuracy of liver biopsy are length and width, with a minimum of 2.5 cm generally required to achieve reproducible sampling. In a study from Paris using the Metavir scoring system, 65% of biopsies 15 mm in length were categorized correctly, which increased to 75% for a 25-mm liver biopsy specimen (187). This same study estimated, using "virtual" biopsy lengths, that biopsy specimens only 4 cm or greater in length would reliably avoid sampling error (187). This is not possible, of course, but it emphasizes that liver biopsy is unlikely to completely overcome the inherent problems of uneven distribution of fibrosis in the liver, combined with inadequate sample size, a problem amplified by increasing reliance on the use of radiologists to obtain liver biopsies using automated devices that are especially narrow. At best, a biopsy captures only 1/50,000th of the liver, and therefore, some sampling error would seem inevitable. Morphometric and computerized systems (187) may yield data that is continuous rather than discontinuous, but if the tissue sample is not adequate, or there is uneven distribution of fibrosis, then these quantitative methods will not enhance the quality of the data obtained.

Immunohistochemical and in situ mRNA hybridization methods to identify specific matrix components or α-smooth muscle actin (to reflect the extent of stellate cell

activation) can be employed for experimental studies, and these methods may have some clinical utility in assessing fibrosis progression (188,189). Semiquantitative or real-time polymerase chain reaction can be used to measure mRNA transcripts for several cytokines and matrix components, for example, PDGF and TGF-β_1. This method has potential utility for analysis of liver biopsy because it may be more sensitive in assessing fibrogenesis than the evaluation of tissue sections stained for ECM. Moreover, even when accurate, this method reflects mRNA levels and not protein levels, and the two may not always correlate.

Noninvasive methods

There is an urgent need for a noninvasive diagnostic procedure for liver fibrosis because this is currently the main limitation of drug testing in clinical trials. Assessment of fibrosis progression can be valuable in HCV for at least four reasons: (a) The actual stage of fibrosis will indicate the likelihood of response to interferon-α or interferon-α/ribavirin because patients with advanced stages of fibrosis (Metavir F3 or F4) generally have a lower response rate to antiviral therapy; (b) if little fibrosis progression has occurred over a long interval, then treatment with antiviral therapy may be deemed as less urgent, and it may be safe to await more effective and/or better tolerated therapy; (c) the approximate time to the development of cirrhosis can be estimated; and (d) as antiviral and antifibrotic therapies are refined, they will be most appropriately tested in "proof-of-principle" trials in patients who are at highest risk for progression in the absence of any treatment. This would not, however, indicate if/when clinical liver failure would eventuate, which may be delayed for up to a decade or more after the establishment of cirrhosis (190). Accurate prediction of fibrosis progression will help identify those patients in whom the efficacy of an antifibrotic drug will be more easily uncovered in a controlled trial compared to placebo-treated control patients who have similar risk factors.

There has been considerable effort to identify serum markers as noninvasive measures for the diagnosis of hepatic fibrosis (191,192,193). Although their accuracy and predictive value are improving, they cannot yet supplant direct analysis of the liver. The *ideal fibrosis marker* is one that is specific, biologically based, noninvasive, easily repeated in all patients, well correlated with disease severity and outcome, and not confounded by comorbidities or drugs. Although this ideal has not yet been reached, progress can be anticipated on the basis of the progress to date and the intense interest in this area.

Serum markers of matrix molecules or modifying enzymes

There has been considerable effort to identify serum markers as noninvasive measures of hepatic fibrosis.

Although their accuracy and predictive value are improving, they cannot yet supplant direct analysis of liver. Thus far, no serum test has emerged as the perfect marker of fibrosis.

Serum markers have some limitations:

- They typically reflect the rate of matrix turnover, not deposition, and therefore tend to be more elevated when there is high inflammatory activity.

In contrast, extensive matrix deposition can go undetected if there is minimal inflammation.

- None of the molecules is liver specific, so concurrent sites of inflammation may contribute to serum levels.
- Serum levels are affected by clearance rates, which may be impaired because of either sinusoidal endothelial cell dysfunction or impaired biliary excretion.
- They are surrogates, not biomarkers.

Currently, there are three commercial serum marker systems that have been most extensively validated, the Fibrospect (Prometheus Laboratories) (191), the FibroTest (marketed in the United States by Labcorp) (193), and the European Liver Fibrosis Study Group panel, utilizing a diagnostic algorithm and assays developed by Bayer-Chiron Diagnostics (192). In addition, a growing list of other noninvasive tests have been developed using a variety of standard serum hematologic or chemistry parameters in varying combinations (194,195,196,197,198).

To date, there are few discernible differences among the three major serum assays, and their value can be summarized as follows: (i) The tests are extremely accurate (>95%) in determining the near absence (F0 or F1) of fibrosis in HCV or presence of cirrhosis, but not intermediate stages; therefore, in select clinical circumstances, the findings from these tests may obviate the need for biopsy or help guide decisions about treatment with antiviral combinations; less is known about their value in patients with other forms of liver disease apart from HCV, but data are beginning to emerge; (ii) they suffer from a variable but significant number of indeterminate outcomes (up to 50% with the FibroTest); (iii) there is no evidence that any test can discern changes in fibrosis content over time in an individual patient; (iv) however, these tests are proving valuable in cohort studies, in which mean changes in serum values among a group of patients will correlate with changes in fibrosis.

Overall, the serum assay approach remains promising, in part because these tests may represent an "integrated" readout of liver activity rather than a minute sampling of the type obtained by conventional liver biopsy. Continued refinements are likely, and they already have established an important niche, especially in patients who have a high risk of complications from liver biopsy or in those with liver disease not under the care of a hepatologist/gastroenterologist (e.g., patients coinfected with HCV/HIV).

Proteomics and glycomics

A different approach is the analysis of patterns of either protein or glycoprotein peaks, as assessed by mass spectroscopy of serum samples (199). These methods clearly represent "surrogate" markers, and in fact, the identities of the peaks are generally not known. Nonetheless, impressive correlations have been reported, particularly a study from Belgium (200) combining serum "glycomics" with one of the noninvasive serum assays, the "FibroTest."

Novel imaging technologies

These strategies currently include efforts to assess the metabolic activity of a cell, to image hepatic scar (201), and/or to directly image or quantify fibrogenic

cells. The best developed among these, the "FibroScan," is a noninvasive device resembling an ultrasound device that measures the elasticity of the liver when applied over the right upper quadrant (202). The test's accuracy is compromised if there is significant overlying adipose tissue, as may be the case in patients with NASH. Recent studies indicate a good ability to discriminate cirrhosis from earlier stages, and its accuracy may be enhanced when combined with other noninvasive serum assays (203). Nonetheless, there is no evidence that changes in fibrosis over time can be detected in individual patients.

In summary, there remains a compelling need for noninvasive markers that accurately reflect the matrix content of the tissue and have better prognostic accuracy than standard clinical and laboratory indices such as Child-Pugh or Model for End-stage Liver Disease (MELD) classifications, which are only applicable to patients with cirrhosis.

Therapy for Hepatic Fibrosis

General considerations

The improved understanding of mechanisms underlying hepatic fibrosis makes effective antifibrotic therapy an emerging reality. However, treatment will remain a challenging task, and thus far no drugs have been approved as antifibrotic agents in humans. Therapies will have to be well tolerated over decades, with good targeting to the liver and few adverse effects on other tissues. Combination therapies may prove synergistic rather than additive, but agents must first be tested individually to establish safety and "proof of principle." It is uncertain whether antifibrotic therapies will require intermittent or continuous administration. For

putative agents, evidence of a direct antifibrotic effect must be established in experimental models rather than by only an indirect effect by abrogating the injury. Candidate therapies must be effective in a liver that is already damaged—as in clinical liver disease—rather than only before the onset of injury. Antifibrotic therapies also carry the theoretical concern that inhibiting the scarring response will prevent the encapsulation of injured regions, leading to extension of tissue damage. In reality, however, antifibrotic therapies only need to downregulate the scar response to be effective, and in patients with cirrhosis it is the scarring, not injury, that usually leads to liver failure.

Antifibrotic therapies—rationale and specific agents

The paradigm of stellate cell activation provides an important framework to define sites of antifibrotic therapy (Table 14.2) (see ref. 20 for review). The rationale is as follows: (a) Cure the primary disease to prevent injury; (b) reduce inflammation or the host response to avoid stimulating stellate cell activation; (c) directly downregulate stellate cell activation; (d) neutralize proliferative, fibrogenic, contractile, and/or proinflammatory responses of stellate cells; (e) stimulate apoptosis of stellate cells; and (f) increase the degradation of scar matrix, either by stimulating cells that produce matrix proteases, downregulating their inhibitors, or by direct administration of matrix proteases.

Table 14.2. Therapeutic Strategies for Hepatic Fibrosis

Reducing injury and inflammation	**Inhibiting properties of activated stellate cells**
Antiviral therapy for viral hepatitis	Antiproliferative
Antihelminthic therapy for schistosomiasis	PDGF receptor antagonists
Chelation/venesection, treatment of metabolic disease	Sodium exchange inhibitors
Angiotensin II type I receptor antagonists, ACE inhibitors	HMG CoA reductase inhibitors
Hepatoprotectants	Plasmin/thrombin receptor antagonists
Caspase inhibitors	Anticontractile
HGF/HGF mimetics	Endothelin/endothelin receptor antagonists
Attenuating stellate cell activation	Nitric oxide donors
Interferon-α	Antifibrogenic
Antioxidants	Collagen synthesis inhibitors
Vitamin E, PDTC	TGF-β inhibitors (soluble receptors, neutralizing antibodies)
Angiotensin II type I receptor antagonists	HGF/HGF mimetics
Cytokine-directed therapy	AT receptor antagonists
TGF-β antagonists	ACE inhibitors
Endothelin receptor antagonists	Integrin

HGF	CTGF/CCN antagonists
PPARγ agonists	Smad 7 agonists
FXR agonists	**Promoting specific apoptosis of hepatic stellate cells**
Aldosterone antagonists	Gliotoxin
Pentoxyphylline	NGF agonists
	TIMP antagonists
	Degrading scar matrix
	Direct collagenase administration
	Inhibitors of transglutaminase or collagen cross-linking
	TIMP antagonists
	TGF-β inhibitors

ACE, angiotensinogen converting enzyme; HGF, hepatocyte growth factor; PDTC, pyrrolidine dithiocarbamate; TGF-β, transforming growth factor β; PPAR, peroxisome proliferators activated receptor; FXR, farnesyl X receptor; PDGF, platelet-derived growth factor; HMG CoA, 3-hydroxy-3-methyl-glutaryl coenzyme A; NGF, nerve growth factor; AT, angiotensin; CTGF, connective tissue growth factor; CCN, *C*ysteine rich 61/*C*onnective tissue growth factor, *N*euroblastoma overexpressed; Smad 7, mothers against DPP homolog 7; TIMP, tissue inhibitor of metalloproteinase.

1. *Cure the primary disease*. The most effective way to eliminate hepatic fibrosis is to clear the primary cause of liver disease. This includes abstinence in alcoholic liver disease, removal of excess iron or copper in precirrhotic genetic hemochromatosis or Wilson disease, clearance of HBV or HCV in chronic viral hepatitis, eradication of organisms in schistosomiasis, or decompression in mechanical bile duct obstruction. Not to be overlooked, weight loss in patients with NASH (204), or even those with HCV who are overweight, may improve histology and is a simple recommendation.

Similarly, reversal of jejunoileal bypass-related hepatic fibrosis and cessation of methotrexate may also prevent progression to cirrhosis. Identification of the pathogenetic mechanisms underlying primary biliary cirrhosis and sclerosing cholangitis could lead to the elimination of bile duct injury and periductular fibrosis.

2. *Reduce inflammation and immune response*. Reduced fibrosis has been reported in patients with HCV successfully treated with pegylated interferon-α and ribavirin (166), presumably through its effect on viral replication and liver injury. Sustained viral clearance will likely be associated with marked regression of fibrosis, so that long-term follow-up of patients successfully cleared of HCV may show more dramatic reversal of disease than at early time points. Importantly, some antifibrotic effect is observed even in the absence of viral clearance (205). In experimental biliary fibrosis interferon-α also reduces fibrosis (206), raising the possibility of a direct antifibrotic mechanism in addition to its antiviral effect.

   A number of agents have anti-inflammatory activity in vitro and in vivo, which may eliminate the stimuli for stellate cell activation. Corticosteroids have been used for decades to treat several types of liver disease, in particular autoimmune hepatitis (207). Their activity is solely as anti-inflammatory agents, with no direct antifibrotic effect on stellate cells. Antagonists to TNF-α or NF-κB modulators have some rationale, as do a growing number of biologically active agents currently used in other chronic inflammatory diseases, in particular inflammatory liver disease. Pentoxyphylline may exert its antifibrotic activity by downregulating TNF-α signaling (208). Other efforts to neutralize inflammatory cytokines include RGD antagonists, which may limit immunologic injury (209,210).

   The renin–angiotensin system may also amplify inflammation through the generation of oxidant stress, and therefore, either angiotensin-converting enzyme antagonists and/or angiotensinogen II type I receptor antagonists may have an anti-inflammatory and antifibrogenic activity (211,212).

   Ursodeoxycholic acid has a beneficial effect on fibrosis in primary biliary cirrhosis (213,214), possibly in part due to its anti-inflammatory activity. Similarly, a nitric oxide–releasing derivative of ursodeoxycholic acid reduces inflammation, fibrosis, and portal pressure in an animal model (215).

   A new class of drugs, broadly referred to as *hepatoprotectants*, are showing considerable promise in preclinical and clinical studies, including HGF, HGF deletion variants, and HGF synthetic mimetics (216,217,218), as well as insulin-like growth factor (219), and a small-molecule caspase inhibitor that improves aspartate aminotransferase levels in patients with chronic HCV and is currently in clinical trials (220). The exact mechanism of HGF's antifibrotic activity is uncertain but may include inhibition of TGF-β_1 activity. Trials in large animals and humans are anticipated, with careful monitoring planned to screen for potential hepatocarcinogenesis because HGF is an hepatocyte mitogen.

3. *Inhibit stellate cell activation*. Reducing the transformation of quiescent stellate cells to activated myofibroblasts is a particularly attractive target, given its central role in the fibrotic response. The most practical approach is to reduce oxidant stress, which is an important stimulus to activation. Antioxidants, including α-tocopherol (vitamin E), suppress fibrogenesis in

some (221), but not all (222), studies of experimental fibrogenesis. Other antioxidants can also reduce stellate cell activation in culture (223), which provides a rationale for antioxidant trials in humans, although, as noted in the preceding text, more potent formulations than those currently available may be required.

Silymarin, a natural flavonoid component of the milk thistle *Silybum marianum*, has sparked interest as a potential antifibrotic therapy. The compound functions as an antioxidant and may decrease hepatic injury by both cytoprotection and inhibition of Kupffer cell function (223,224). A single human trial in patients with cirrhosis has reported a slight survival advantage in patients with alcoholic cirrhosis who have Child-Pugh A disease (225), but larger, carefully controlled studies are under way to definitively assess its efficacy.

The cytokine interferon-γ has inhibitory effects on stellate cell activation in animal models of fibrosis (226). However, a clinical trial of interferon-γ did not show the expected antifibrotic benefit in patients with HCV, possibly because only patients with advanced fibrosis were enrolled and the treatment period (1 year) may have been too short.

Interferon-α, in addition to its antiviral effect, can downregulate stellate cell activation and fibrogenesis directly through well-defined molecular pathways, and suppress experimental fibrosis (227). Current long-term low-dose interferon-α trials in HCV nonresponders, including Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) (228) are based in part on this mechanism of action, and if these trials succeed the drug may emerge as the first *bona fide* antifibrotic in humans. Inhibition of collagen gene expression in experimental models may also be accomplished using synthetic oligonucleotides to inhibit collagen promoter activity (229).

Great excitement was generated by an uncontrolled trial of interleukin 10, which reported an antifibrotic effect in patients with advanced HCV (230). The finding was particularly exciting because of parallel studies in animals and cultured stellate cells suggesting a direct antifibrotic activity of the agent (231,232,233,234). However, a controlled clinical trial showed no benefit, possibly because the drug markedly increased HCV viral load (235).

PPARγ nuclear receptors are expressed in stellate cells, and synthetic PPARγ ligands (thiazolidinediones) downregulate stellate cell activation (116,236). Given

their widespread use in diabetes, second- and third-generation thiazolidinediones (i.e., lacking the hepatotoxicity seen with first-generation agents such as troglitazone) are now being tested in clinical trials for both NASH and other fibrotic liver diseases. FXR ligands appear to have similar effects and small-molecule agonists are under study in preclinical models with evidence of antifibrotic activity (120).

Leptin, produced by activated stellate cells (237), not only affects lipid metabolism but also directly influences wound healing. In fact, animals deficient in leptin have reduced hepatic injury and fibrosis (238,239). On the basis of this finding, the discovery of adiponectin, a natural counter-regulator to leptin, may lead to the use of this agent in fibrosis, particularly in association with NASH (147,148).

Progress in understanding transcriptional regulation has offered the opportunity to block stellate cell activation by inhibiting the activity of histone deacetylases (HDACs), enzymes critical for modifying chromatin during gene transcription (240). Highly specific HDAC inhibitors offer the potential for selectively blocking stellate cell activation with tolerable safety and good efficacy (241), but none has reached clinical use. Similarly, modulation of intracellular proteins including transcription factors remains an elusive target for antifibrotic therapy.

Herbal therapies and products derived from natural compounds that are commonly used in the Far East are increasingly being tested under controlled, scientifically rigorous conditions (242) and some show promise of efficacy, in particular Sho-saiko-to (243), *Salvia miltiorrhiza* (244), and a green tea polyphenol (245).

4. *Neutralize proliferative, fibrogenic, contractile, and/or proinflammatory responses of stellate cells*. Significant advances in growth factor biology will benefit the treatment of hepatic fibrosis through the development of antagonists to cytokines and their receptors. In particular, many proliferative cytokines including PDGF, FGF, and TGF-α signal through tyrosine kinase receptors, inhibitors of which are already undergoing clinical trials in other tissues (246). Because the intracellular signaling pathways for these receptors are well understood, inhibitors to signaling models are being explored in vivo or in cultured stellate cells, including γ-linoleic acid and lipoxygenase inhibitors (247), and PPARγ pathways (see preceding text). Others include inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (248); pentoxyphylline, which inhibits PDGF receptor signaling (249), and compounds that elevate intracellular cyclic adenosine monophosphate levels (250) or block ion transporters, including pirfenidone (70,251).

   The recent success in developing Gleevec, a safe, effective, small-molecule tyrosine kinase antagonist in human leukemia and mesenchymal cell tumors (252,253), augurs well for the potential of this approach in other indications, including liver fibrosis. In fact, Gleevec is antifibrotic in experimental liver fibrosis (254). Other orally available, low-molecular-weight small molecules are under development to block cytokine receptor or intracellular signaling. One such compound is a selective inhibitor of Rho-mediated focal adhesions, which can reduce experimental liver fibrosis (255). Antisense to PDGF B chain also blocks experimental hepatic fibrosis (256). Because siRNA technology is increasingly becoming clinically applicable, this approach may merit further evaluation.

   Inhibition of matrix production has been the primary target of most antifibrotic therapies to date. This has been attempted directly by blocking matrix synthesis and processing, or indirectly by inhibiting the activity of TGF-β_1, the major fibrogenic cytokine. Inhibitors of collagen synthesis such as HOE 077, which blocks the enzyme prolyl hydroxylase, were among the first antifibrotic compounds tested in liver diseases, but success with this agent has been modest. The emerging importance of translational regulation of collagen gene expression (87,257,258,259) could lead to specific translational inhibitors with therapeutic value. Colchicine generated excitement at one time because of its apparent efficacy in a small group of patients (260); however, a more recent study in alcoholic cirrhosis showed

no benefit (261).

TGF-β antagonists are being extensively tested because neutralizing this potent cytokine would have the dual effect of inhibiting matrix production and accelerating its degradation (see ref. 78). Animal and culture studies using soluble TGF-β receptors or other means of neutralizing the cytokine, including monoclonal antibodies and protease inhibitors to block TGF-β activation, have established proof of principle (20,262,263). Concerns that inhibiting TGF-β may alter hepatocellular growth or apoptosis will need to be considered as these antagonists reach clinical trials, but in other tissues there is great promise for this approach. A number of even newer TGF-β antagonists are also being developed and may undergo testing soon. These could include recombinant Smad 7, which antagonizes TGF-β activity in stellate cells (264).

Rapamycin, an immunosuppressive drug used after liver transplantation has the added benefit of inhibiting stellate cell proliferation (265), which could attenuate the accelerated fibrosis progression in patients with recurrent HCV; however, the enthusiasm for using rapamycin has been tempered by a reported increase in hepatic artery thrombosis (266).

Relaxin, a natural peptide hormone that mediates parturition, has been developed as an agent that decreases collagen synthesis by stellate cells and

increases matrix degradation in vitro and in vivo (267). Stellate cells also express relaxin receptors (268), which might represent an attractive target for antagonism.

Because endothelin-1 is an important regulator of wound contraction and blood flow regulation mediated by stellate cells, antagonists have been tested as both antifibrotic and portal hypotensive agents. Bosentan, a mixed endothelin A and B receptor antagonist, has antifibrotic activity and reduces stellate cell activation in experimental hepatic fibrosis (269). This and other endothelin antagonists remain attractive drug development targets (270). Alternatively, the delivery of nitric oxide to an injured liver may have the same therapeutic effect as inhibiting endothelin-1 (215,271).

Halofuginone, an anticoccidial compound, has antifibrotic activity by blocking collagen expression and has been used in a number of models of tissue fibrosis, including the liver (272).

The potential utility of retinoids (vitamin A) as antifibrotic therapy has been limited by inadequate knowledge about their regulatory role in stellate cell activation and by toxicity concerns. Although stellate cells export retinoid as they activate, it does not follow that restoration of cellular retinoid will prevent activation. In fact, some retinoids may accelerate fibrosis by augmenting membrane injury as in hypervitaminosis A (273).

5. *Stimulate stellate cell apoptosis*. Attention is increasingly focused on how liver fibrosis regresses, and in particular, the fate of activated stellate cells as fibrosis recedes. Mounting evidence indicates that both reversal of the activated stellate cell phenotype and apoptosis are possible. In particular, as liver fibrosis is decreased, there is selective cell death of activated stellate cells (45). This exciting observation has led to animal studies using gliotoxin, which provokes selective apoptosis of stellate cells in culture and

in vivo, leading to reduced fibrosis (274,275). A TIMP-1 neutralizing antibody has antifibrotic activity in experimental liver fibrosis (276). Similarly, inhibition of Iκβ, whose net effect is to increase NF-κB signaling in stellate cells, may accelerate apoptosis (175). Apoptosis can also be provoked by the disruption of integrin-mediated adhesion (210) or through the use of tumor necrosis factor-α-related apoptosis-inducing ligands (TRAILs) (277). Stellate cells contain several families of apoptotic mediators, including Fas/FasL, TNF receptors, nerve growth factor receptors (278), and Bcl/Bax, so that additional targets to promote apoptosis will likely be exploited in the future (279).

6. *Increase the degradation of scar matrix*. This component of treatment is very important because antifibrotic therapy in human liver disease will need to provoke resorption of existing matrix in addition to preventing deposition of new scar. As noted in the preceding text, TGF-β antagonists have the advantage of stimulating matrix degradation by downregulating TIMPs and increasing net activity of interstitial collagenase (see ref. 35 for review). Retinoids may also stimulate matrix degradation but concerns over toxicity limit their utility. Relaxin can directly increase matrix degradation (280).

   Direct expression of metalloproteinases in animal models of hepatic fibrosis has begun to confirm that matrix can be resorbed by the expression of exogenous enzymes (281,282). Although this may seem impractical in humans, the data establishes the important proof of principle that matrix is responsive to degradation. Moreover, an experimental study has affirmed the importance of matrix degradation in the regression of hepatic fibrosis by demonstrating that a genetically altered mouse expressing mutant collagen resistant to degradation displays delayed regression of fibrosis after liver injury (283).

Future Prospects

Continued progress can be anticipated in unraveling the molecular regulation of fibrosis and its treatment. Rapid advances in gene therapy, tissue-specific targeting, and high-throughput small-molecule screening of cytokine inhibitors are likely to benefit the diagnosis and therapy of hepatic fibrosis. Methods have been developed for stellate cell–specific targeting in animal models (284), which could lead to successful directed therapy to minimize toxicity of antifibrotics and for use as novel diagnostics. New insights into the regulation of growth and apoptosis could have direct implications for stellate cell behavior in liver injury. Sequencing of the human genome and use of high throughput methods begun to yield genetic polymorphisms that predict the rate of fibrosis and patterns of multi-gene expression that have clinical or therapeutic implications, respectively. Additionally, there is tremendous interest in herbal and natural antifibrotic remedies, particularly in the Far East, where many such compounds are undergoing clinical trials. Accelerating progress is certain, once methods of noninvasive diagnosis are established that enable rapid assessment of fibrosis in clinical trials, and ultimately in clinical practice.

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Chapter 15

Portal Hypertension and Nonsurgical Management

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Key Concepts

- Portal hypertension is the most common and lethal complication of chronic liver diseases; it is responsible for the development of gastroesophageal varices, variceal hemorrhage, ascites, renal dysfunction, portosystemic encephalopathy, hypersplenism, and hepatopulmonary syndrome.
- Portal hypertension is defined by a pathologic increase in portal pressure, in which the pressure gradient between the portal vein and inferior vena cava (the portal pressure gradient [PPG]) is increased above the upper normal limit of 5 mm Hg. Portal hypertension becomes clinically significant when the PPG increases above the threshold value of 10 mm Hg (e.g., formation of varices) or 12 mm Hg (e.g., variceal bleeding, ascites). PPG values between 6 and 10 mm Hg represent subclinical portal hypertension.
- PPG is determined by the product of blood flow and vascular resistance within the portal venous system. Portal hypertension is initiated by an increased resistance to portal blood flow and aggravated by an increased portal venous inflow. The site of increased resistance to portal blood flow is the basis for the classification of portal hypertension: Prehepatic (e.g., portal vein thrombosis), intrahepatic (e.g., cirrhosis), and posthepatic (e.g., hepatic vein thrombosis, heart disease).
- Increased resistance in cirrhosis represents not only disruption of the liver's vascular architecture by liver disease but also a dynamic component resulting from the active contraction of vascular smooth muscle cells, myofibroblasts, and hepatic stellate cells. Active contraction is caused by decreased production of vasodilators such as nitric oxide (NO) and by increased release of endogenous vasoconstrictors. Increased hepatic vascular tone is the basis for the use of vasodilators to treat portal hypertension in cirrhosis.
- Portal inflow is increased by splanchnic vasodilatation, which is caused by an increased release of local endothelial factors and humoral vasodilators. Splanchnic vasodilatation can be counteracted with vasoconstrictors and β-blockers, which is why these drugs are used to treat portal hypertension.
- Portal pressure is most commonly assessed clinically by measuring the hepatic venous pressure gradient (HVPG), which is the difference between the wedged hepatic venous pressure (WHVP) and free hepatic venous pressure (FHVP), at hepatic vein catheterization. The HVPG accurately reflects the portal pressure in both alcoholic and viral cirrhosis. HVPG has to be above 10 mm Hg for varices to develop and above 12 mm Hg for variceal bleeding. The threshold values define "clinically significant portal hypertension."

- Bleeding from ruptured esophageal or gastric varices is a major complication of portal hypertension and a frequent cause of death. Approximately 40% of patients with compensated cirrhosis have varices at the time of diagnosis. The rate of formation of varices is approximately 5% per year, so varices develop in most patients during long-term follow-up. The risk of bleeding increases as the pressure and size of the varices increase and as the thickness of the variceal wall decreases. These parameters determine the tension of the variceal wall; rupture and bleeding occur when wall tension increases above the elastic limit of the varices.
- Most drugs used to treat portal hypertension are vasoconstrictors, which act primarily by reducing portal and collateral blood flow. This group includes vasopressin, terlipressin, the somatostatins, propranolol, nadolol, and other β-blockers.
- Vasodilators that decrease the portal pressure include isosorbide-5-mononitrate (IMN), which acts as an NO donor, and adrenergic antagonists, such as clonidine, prazosin, and carvedilol. A common problem with vasodilators is that they cause arterial hypotension, which in turn may enhance sodium retention and aggravate renal dysfunction in patients with cirrhosis and ascites.
- In combination therapy, a vasoconstrictor and a vasodilator are administered together. The combination prevents most of the adverse effects of the vasodilator and enhances the fall in portal pressure caused by the reduction in blood flow induced by the vasoconstrictor. Drug combinations with proven clinical efficacy are vasopressin plus nitroglycerin and propranolol or nadolol plus IMN.
- Continued drug therapy with propranolol or nadolol is highly effective in preventing first bleeding in patients with varices. This treatment should be given to all patients with medium or high variceal bleeding risk (patients with medium or large varices or those with small varices and red color signs or with poor liver function—Child-Pugh B and C) who have no contraindications to β-blockers. Therapy should be maintained indefinitely and is relatively well tolerated; the rate of discontinuation because of side effects or poor tolerance is 15%. Patients with high-risk varices and contraindications to β-blockers should be treated with endoscopic band ligation.
- During acute variceal hemorrhage, general supportive therapy (including antibiotic prophylaxis of bacterial infections and a very conservative use of blood transfusion) is essential to reduce mortality. The prognosis is worse in patients with advanced liver failure or with early (first-week) rebleeding. Terlipressin has proved effective as a first-line treatment in arresting variceal bleeding and reducing mortality from variceal hemorrhage. Somatostatin is also effective. Drug therapy has been shown to be as effective as and safer than emergency endoscopic therapy. Drug therapy should be started early on arrival at the emergency room or even during transfer to the hospital. Endoscopic therapy is established after confirming that esophageal varices are indeed the source of bleeding (66% of bleeding episodes in cirrhosis). Endoscopic band ligation is the endoscopic treatment of choice for acute esophageal variceal bleeding; however, endoscopic injection sclerotherapy can be used in the acute setting if endoscopic band ligation is technically difficult. It is recommended to maintain drug therapy for 2 to 5 days (even if the bleeding is apparently controlled by endoscopic therapy). Endoscopic variceal obturation with tissue adhesives is recommended for acute fundal gastric variceal bleeding.
- Failures of medical treatment should be managed aggressively with emergency surgery or transjugular intrahepatic portosystemic shunting (TIPS), preferably using expanded polytetrafluoroethylene (ePTFE) covered stents. Because of higher rates of morbidity and mortality, rescue derivative surgery should only be considered in low-risk patients (Child-Pugh score <8).

- Patients who survive an episode of variceal bleeding are at high risk for rebleeding. Medical treatment with β-blockers ± isosorbide mononitrate is as effective as endoscopic band ligation in preventing rebleeding. The best results are obtained when HVPG is reduced by at least 20% of the baseline value or below 12 mm Hg. The combination of IMN and propranolol or nadolol significantly increases the number of patients in whom the target reduction in portal pressure is achieved.
- Combination of β-blockers and band ligation is probably the best treatment option, especially in patients who have bleeding while under either therapy alone. Patients who rebleed despite combined endoscopic and pharmacologic treatment may be treated by TIPS or surgical portosystemic shunt; TIPS is the only option in nonsurgical candidates. All Child-Pugh class C patients should be considered for liver transplantation.

Portal hypertension is a common clinical syndrome, defined by a pathologic increase in the portal venous pressure, in which the pressure gradient between the portal vein and inferior vena cava (IVC) (portal perfusion pressure of the liver [PPG]) is increased above normal values (1 to 5 mm Hg). When the PPG rises above 10 to 12 mm Hg, complications of portal hypertension can arise (1,2,3,4). Therefore, this value represents the threshold for defining portal hypertension as being clinically significant.

The importance of this syndrome is defined by the frequency and severity of complications: Massive upper gastrointestinal bleeding from ruptured gastroesophageal varices and portal hypertensive gastropathy (PHG), ascites, renal dysfunction, hepatic encephalopathy, arterial hypoxemia, disorders in the metabolism of drugs or endogenous substances that are normally eliminated by the liver, bacteremia, and hypersplenism (5). These complications are major causes of death and the main indications for liver transplantation in patients with cirrhosis.

This chapter provides a background on the most important aspects of the pathogenesis, evaluation, and treatment of portal hypertension.

Etiology of Portal Hypertension

Portal hypertension can be caused by any disease interfering with blood flow at any level within the portal venous system. According to anatomic location, the diseases causing portal hypertension are classified as prehepatic (diseases involving the splenic, mesenteric, or portal veins), intrahepatic (acute and chronic liver diseases), or posthepatic (diseases interfering with the venous outflow of the liver) (5) (Table 15.1). Cirrhosis of the liver is by far the most common cause of portal hypertension in the world, followed by hepatic schistosomiasis. All other causes account for less than 10% of cases, which is why these cases are sometimes referred as *noncirrhotic portal hypertension*.

Prehepatic Portal Hypertensive Syndromes

Prehepatic portal hypertension is an infrequent condition in which increased portal pressure is caused by obstruction of the portal venous tree before it enters the liver. The site of obstruction may be limited to the splenic or mesenteric veins, but most commonly the portal vein is involved. Obstruction may be caused by congenital abnormalities or, more usually, by various underlying diseases that affect a part or all of the vessels of the portal venous system. In all these conditions, it is essential to obtain accurate anatomic information about the site and extent of the obstruction because it may affect therapeutic decisions.

Portal vein thrombosis

Portal vein thrombosis (PVT) is the most frequent cause of prehepatic portal hypertension. In children, a history consistent with omphalitis or umbilical catheterization can be often obtained. In adults, thrombophilic factors have been identified in approximately 60% and local

factors in 40% of patients with PVT (6,7). Several of these risk factors are frequently associated (8). However, despite a complete thorough investigation, up to 30% of episodes of PVT remain idiopathic. The spontaneous formation of erythroid colonies in culture increases the likelihood of the diagnosis of latent myeloproliferative disorders not detected by conventional criteria (9). This may be further facilitated by the recent identification of the *Janus kinase 2* (*JAK2*) gene mutation as a molecular marker of myeloproliferative disorders (10,11). Local factors include local inflammatory/infectious disorders (e.g., pancreatitis, cholecystitis, cholangitis, and appendicitis), local injury to the portal venous axis (e.g., splenectomy, abdominal trauma, surgical portacaval shunting, and other intra-abdominal surgical procedures). Other associated with PVT are the use of oral contraceptives, pregnancy, and inflammatory and malignancy states (6). The presence of these factors do not exclude the presence of systemic risk factors (8).

Table 15.1. Etiology of Portal Hypertension

Prehepatic
Splenic vein thrombosis
Portal vein thrombosis
Congenital stenosis of the portal vein
Extrinsic compression of the portal vein
Arteriovenous fistulae (splenic, aortomesenteric, aortoportal, and hepatic artery–portal vein)[a]
Intrahepatic
Partial nodular transformation
Nodular regenerative hyperplasia
Congenital hepatic fibrosis
Peliosis hepatis
Polycystic disease
Idiopathic portal hypertension
Hypervitaminosis A
Arsenic, copper sulfate, and vinyl chloride monomer poisoning
Sarcoidosis
Tuberculosis
Primary biliary cirrhosis
Schistosomiasis
Amyloidosis
Mastocytosis
Rendu-Osler-Weber syndrome
Liver infiltration in hematologic diseases
Acute fatty liver of pregnancy
Severe acute viral hepatitis
Chronic active hepatitis
Hepatocellular carcinoma
Hemochromatosis
Wilson disease

	Hepatic porphyrias
	α_1-Antitrypsin deficiency
	Cyanamide toxicity
	Chronic biliary obstruction
	Cirrhosis from hepatitis B and C virus infection
	Alcoholic cirrhosis
	Alcoholic hepatitis
	Veno-occlusive disease
Posthepatic	
	Budd-Chiari syndrome
	Congenital malformations and thrombosis of the inferior vena cava
	Constrictive pericarditis
	Tricuspid valve diseases

[a]This is the only instance in which portal hypertension is not initiated by an increased resistance to portal blood flow.

Cirrhosis may be considered as a local factor promoting PVT. The prevalence of PVT in patients with cirrhosis without associated hepatocellular carcinoma ranges between 0.5% and 26% (12). The concomitant existence of thrombophilic factors in patients with cirrhosis has been suggested to be a relevant risk factor for PVT (13). Other factors that are involved are endoscopic sclerotherapy, the use of vasoactives drugs, past history of variceal bleeding, and a low platelet count (12).

In most adult cases, chronic PVT is diagnosed after an episode of bleeding from ruptured esophageal varices or during routine evaluation (e.g., abdominal evaluation, ultrasonography, or upper endoscopy), whereas in children it is more frequently diagnosed after detecting splenomegaly. Variceal bleeding is much better tolerated in patients with prehepatic portal hypertension than in those with cirrhosis because the former do not have liver failure. Ascites and encephalopathy may develop following bleeding, although infrequently. In these cases, a diagnosis of concomitant liver disease should be considered.

Portal biliopathy, defined as the abnormalities of the extrahepatic and intrahepatic bile ducts observed by imaging techniques in patients with portal cavernoma (14), has been identified in over 80% of patients after long-standing portal vein obstruction (15). It is attributed to biliary compression by the peribiliary collaterals composing the cavernoma. Despite the high rate of biliary tract abnormalities, clinical consequences are infrequent (16), although increased serum alkaline phosphatase levels can be commonly found. However, some cases may develop severe, life-threatening manifestations (e.g., cholecystitis, cholangitis, and obstructive jaundice).

In some rare cases, the diagnosis is made in the acute phase of the disease, and the manifestations of acute portal vein obstruction are mixed with those of the initiating factor, such as recent surgery or abdominal infection. Patients may present with abdominal pain and fever. Diarrhea, ileus, and gastrointestinal bleeding resulting from intestinal infarction may be present. The severity of these symptoms is variable. The clinical suspicion of portal thrombosis should prompt ultrasonographic examination of the portal system. The identification of solid echoes within the portal vein and the absence of flow signals when pulsed Doppler equipment is used confirm the diagnosis. Echo enhancement may be useful. Magnetic resonance imaging (MRI) and helical computed tomographic (CT) scan are very useful, although not mandatory if ultrasonography clearly

shows PVT. When these noninvasive techniques do not demonstrate the site and extent of PVT, other diagnostic procedures, such as angiography, should be used. Angiography is also used in select cases, especially when a surgical approach is being considered. Retrograde wedged hepatic venography with the use of carbon dioxide as contrast agent may also be useful.

In the chronic phase, PVT is associated with the development of new vascular channels that bypass the obstruction, so that the portal vein takes on the appearance of a cavernoma. This is best shown by angiography but is also recognized on abdominal ultrasonography.

Patients with portal vein obstruction exhibit the same hemodynamic abnormalities as those with any other type of portal hypertension. These patients exhibit a hyperdynamic state, with increased blood volume and cardiac output and reduced arterial pressure and peripheral vascular resistance (17). The splanchnic circulation is also hyperdynamic, with increased inflow of blood into the portal venous system. Serum biochemistry shows either no abnormalities or a mild elevation in the concentration of globulins, and the hematologic examination may disclose a variable degree of hypersplenism, evidenced by leukopenia, thrombocytopenia, and anemia.

In acute PVT spontaneous recanalization is extremely infrequent. However, over 50% of patients receiving early anticoagulation achieve portal recanalization (18). Therefore, after the diagnosis of acute PVT early anticoagulation is mandatory. It is recommended to maintain patients on anticoagulation for at least 6 months (18). It, however, seems wise to maintain long-term anticoagulation in patients with an identified underlying prothrombotic disorder, with recurrent episodes of thrombosis, or a familial history of venous thrombosis (8). This is also true for patients with chronic PVT where, although recanalization is not expected to occur, anticoagulation has been shown to prevent the progression and recurrence of thrombosis without increasing the risk or severity of gastrointestinal bleeding (19), provided adequate treatment to prevent variceal bleeding is also established.

Direct thrombolytic therapy through a catheter introduced into the portal vein (either percutaneously or by a transjugular approach) may be useful in some cases of PVT. However, there are no studies evaluating whether this invasive approach offers advantages over early anticoagulation. Our own experience with direct thrombolysis has been disappointing.

In the absence of large specific studies, pharmacologic treatment with nonselective β-blockers, endoscopic band ligation, or the combination of both methods is used for preventing first variceal bleeding (8). Acute episodes of gastrointestinal hemorrhage are treated in the same manner as in patients with cirrhosis. Percutaneous transhepatic stenting of portal cavernoma is sometimes possible in instances of chronic PVT, from either a transjugular or a transhepatic approach and should be attempted if angiography suggests that the cavernoma has one or several vessels greater than 2 mm in diameter. Splenectomy and derivative surgery are strongly discouraged in children and should never be attempted in patients who have not bled. The best operation is a portoportal interposition bypass graft shunt, connecting a potent mesenteric/splenic vein with the intrahepatic portal vein, which is usually not thrombosed. Distal splenorenal shunt (DSRS) is the second choice when the splenic vein is patent. Mesocaval graft shunts are sometimes possible. Other procedures, such as esophageal transection or splenectomy and ligation of the varices, are less useful because of the high frequency of gastric varices and the high index of late rebleeding secondary to recurrent varices.

Splenic vein thrombosis

The main causes of isolated splenic vein thrombosis are chronic pancreatitis and carcinoma of the pancreas (20). Occasionally, splenic vein thrombosis has been

described in association with retroperitoneal infection or retroperitoneal fibrosis.

Usually, splenic vein obstruction is diagnosed after an episode of bleeding related to gastric varices. Physical examination reveals an enlarged spleen; the results of biochemical determinations are usually normal, whereas hematologic parameters may indicate hypersplenism. Gastric varices are a prominent (if not the only) endoscopic finding in these patients. The diagnosis is established by ultrasonography and splenic artery angiography. It should be noted that nonvisualization of the splenic vein on splanchnic angiography is not unequivocal evidence of splenic vein thrombosis. False-positive results are frequent in patients with marked splenomegaly. In these cases, MRI can be helpful.

Unlike subjects with portal thrombosis, patients with isolated splenic vein obstruction should be treated surgically because splenectomy is a curative operation and not associated with high rates of morbidity and mortality. However, patients without previous bleeding must be informed in detail about the risks and the long-term care that they will require after splenectomy before they opt for surgery.

Congenital stenosis of the portal vein

It is important to consider congenital stenosis of the portal vein in children with prehepatic portal hypertension. The stenosis may be found at any point along the portal vein, but the hepatic hilus and the middle of the trajectory of the vein are particularly common locations. As in other patients with prehepatic portal

hypertension, the disease is usually diagnosed after an episode of gastrointestinal bleeding from ruptured gastroesophageal varices. The physical examination may show splenomegaly and subcutaneous collateral vessels. Parameters of liver function remain within the normal range, and ultrasonography shows patency of the portal vein, thereby suggesting a false diagnosis of intrahepatic portal hypertension. However, liver histology is normal, as are hepatic venous pressures. Careful ultrasonographic examination of the portal vein and analysis of the venous phase of splanchnic angiography may reveal the stenosis, which can then be confirmed by measuring the venous pressure along the portal vein and demonstrating a pressure gradient at the location of the stenosis. These children can be managed medically, by transluminal balloon angioplasty (with or without stenting), or surgically.

Extrinsic compression of the portal vein

Portal hypertension resulting from compression of the portal vein by a mass or a process located in the vicinity of its trajectory is an unusual condition. However, the splenic vein may be compressed by benign or malignant disease of the pancreas or by retroperitoneal fibrosis.

Partial nodular transformation of the liver is an infrequent disease of unknown origin characterized by the appearance of large nodules (up to 8 cm in diameter) in the hilar area of the liver. Serum liver chemistries are usually normal, and the condition is diagnosed either after an episode of bleeding from esophageal varices or during the diagnostic workup of an enlarged spleen. Pathologic examination shows the hilar nodules, which are composed of normal hepatocytes with minimal fibrosis, and a normal appearance of the surrounding liver. The nodules compress the portal vein, which on portography shows a characteristic smooth narrowing at the hepatic hilum.

Arteriovenous fistulae in the portal venous system

Arteriovenous fistulae in the spleen and splanchnic vascular bed may present with portal hypertension, ascites, and varices. Approximately one third of patients have abdominal pain, and in some of these cases, it is possible to hear an abdominal bruit. The location of arteriovenous fistulae may be intrahepatic or extrahepatic (e.g., in the

spleen or portal vein). They may be of congenital origin, associated with the Rendu-Osler-Weber syndrome, or caused by trauma. Intrahepatic arterioportal fistulae may be caused by liver biopsy or other invasive procedures. In other cases, fistulae are observed within a hepatocellular carcinoma. In patients with cirrhosis, the development of an intrahepatic arteriovenous fistula may markedly aggravate portal hypertension. In rare cases, an aneurysm of the hepatic artery may rupture into the portal vein and give rise to a fistula and portal hypertension. Aorta–portal fistulae or intrasplenic arteriovenous fistulae are infrequent complications of abdominal wounds.

Treatment of these patients by percutaneous arterial embolization is usually feasible. Otherwise, surgical ligation of the fistula may be required. These procedures are followed by total or partial normalization of the portal pressure. Arteriovenous fistulae increase the portal blood flow, and this may be the unique situation in which the portal pressure increases without an increased resistance to portal blood flow. However, evidence suggests that secondary "hepatoportal sclerosis," with fibrosis of portal radicles and thickening and sclerosis of the portal veins, may develop in response to an "arterialized" portal system. This is probably the reason why in some of these patients, portal hypertension is not entirely corrected despite surgical closure of the fistula (21).

Splenomegaly

Splenomegaly in the setting of hematologic or deposit diseases, such as leukemia, lymphoma, polycythemia vera, and myelophthisis with myeloid metaplasia, may be associated with complications of portal hypertension, including ascites and variceal hemorrhage, which were thought to be a consequence of an increase in portal blood flow (22). However, in most cases, the primary disease also infiltrates the liver, and portal hypertension is mostly caused by an increased resistance to portal blood flow.

Intrahepatic Causes of Portal Hypertension

Intrahepatic causes of portal hypertension have been classified according to the anatomic zone of obstruction to portal blood flow within the liver and according to the results of hepatic vein catheterization (see subsequent text). Hence, these syndromes are divided into presinusoidal (with normal "wedged" pressure), sinusoidal (with increased wedged pressure and normal "free" pressure), and postsinusoidal categories (with increases in both wedged hepatic venous pressure [WHVP] and free hepatic venous pressure [FHVP]). Some diseases cause lesions at several sites.

These liver diseases (Table 15.1) are fully discussed in other chapters. The specific aspects related to portal hypertension are briefly reviewed here.

Schistosomiasis

Portal hypertension is the consequence of a granulomatous reaction to the deposition of parasite eggs in the portal venules. The inflammatory response leads

to fibrosis and obliteration of the portal venules, with manifestations of portal hypertension in the absence of significant hepatocellular injury. As the disease progresses, fibrosis extends into the sinusoids, and the hemodynamic pattern and clinical course then resemble those of liver cirrhosis.

Sarcoidosis

Portal hypertension is an infrequent manifestation of hepatic sarcoidosis. Sarcoid granulomas frequently localize in the portal areas of liver lobules and injure portal venules. Early disease is predominantly presinusoidal, with normal sinusoidal pressure, whereas advanced disease leads to fibrosis and cirrhosis.

Myeloproliferative diseases

Myeloproliferative diseases may cause intrahepatic portal hypertension as malignant cells directly infiltrate the liver.

Nodular regenerative hyperplasia

Nodular regenerative hyperplasia is a nonspecific reaction to a variety of injuries (see Chapter 40). It is characterized by a nodular transformation of the hepatic parenchyma without fibrous tissue between the nodules. The cause of nodular regenerative hyperplasia seems to be heterogeneity of the blood supply. Whereas acini with normal or increased blood flow undergo hypertrophy, others, with decreased blood flow, undergo atrophy. This process may be the result of diseases that decrease the blood flow in the portal venules, in most cases as a result of portal thrombosis, and is probably the main mechanism leading to portal hypertension in this condition. Compression of the portal venous tract by the nodules may also play a role.

Primary biliary cirrhosis

The development of portal hypertension in the initial stages of primary biliary cirrhosis (before the development of cirrhosis) is thought to be caused by injury of portal venules at the portal tracts, which promotes a presinusoidal type of portal hypertension. Progression of the disease, with the development of fibrous tracts, adds a sinusoidal component. This is always present when the disease reaches stage IV.

Idiopathic portal hypertension

Idiopathic portal hypertension is known by different names, including *Banti's syndrome, noncirrhotic portal fibrosis*, and *hepatoportal sclerosis*. The syndrome is recognized predominantly in Japan and India. In India, up to 30% of patients with portal hypertension have this disease, with a male predominance noted.

Variceal bleeding is the main clinical manifestation of idiopathic portal hypertension; ascites, encephalopathy, and other signs of liver failure are encountered only in the late stages of the disease. Variceal hemorrhage is usually well tolerated, and endoscopic techniques or portacaval anastomosis is associated with low rates of rebleeding and death.

The etiology of idiopathic portal hypertension has not been explained satisfactorily. Exposure to toxins (arsenic and others) or infectious agents has been postulated, without confirmation. It has been suggested that idiopathic portal hypertension and idiopathic portal thrombosis may be part of the same spectrum, in which the lesion of the portal branches would affect the distal intrahepatic part of the portal tree in idiopathic portal hypertension and the more proximal branches in portal thrombosis.

In idiopathic portal hypertension, the liver surface may be pseudonodular. This fact should be taken into account so that the disease is not confused with liver cirrhosis. Patients with idiopathic portal hypertension must be distinguished by liver biopsy from patients with well-compensated cirrhosis.

Toxicity caused by vinyl chloride, arsenic, vitamin A, mercaptopurine, azathioprine, thioguanine, and other agents

A variety of drugs and chemicals have been associated with the development of portal hypertensive liver disease in the absence of cirrhosis. Among these, arsenic is a well-known cause of portal hypertension. Probably a consequence of vascular injury, a wide spectrum of arsenic-related lesions is recognized: Angiosarcoma, perisinusoidal fibrosis, peliosis hepatis in association with angiosarcoma, and veno-occlusive disease with centrilobular sinusoidal dilatation and perisinusoidal fibrosis. Other etiologic agents in addition to arsenic include vinyl chloride, vitamin A, busulfan, chlorambucil, mercaptopurine, and azathioprine. They all cause lesions with many similarities to

those of idiopathic portal hypertension.

Acute and fulminant viral hepatitis

Portal hypertension with all its complications has been described in severe acute hepatitis and fulminant hepatic failure of various causes. A significant correlation has been demonstrated between the severity of portal hypertension (assessed by hepatic vein catheterization) and the severity of hepatitis (indicated by encephalopathy, elevation of serum bilirubin and albumin levels, and coagulopathy). Histologically, portal hypertension seems to correlate with the degree of collapse of the sinusoids and reduction in the intrahepatic vascular space resulting from hepatic necrosis.

Acute fatty liver of pregnancy

A moderate degree of portal hypertension is frequently found in patients with acute fatty liver of pregnancy. It is thought to be caused by compression of the sinusoids by the microvesicular steatosis.

Posthepatic Portal Hypertension Syndromes

Posthepatic obstruction can take place in the IVC or at the level of superior vena cava or right atrium. It may occur in any disease in which right-sided heart pressures are increased.

Inferior vena cava obstruction

The causes of mechanical obstruction of the IVC include venous thrombosis, tumor, cysts, and abscesses. Membranous obstruction may be caused by a fibrous web located above the hepatic veins. The clinical presentations of these extrahepatic syndromes overlap with those of intrahepatic postsinusoidal syndromes, such as Budd-Chiari syndrome or veno-occlusive disease with congestive hepatomegaly, which may be painful and associated with ascites and variceal bleeding. The presence of venous collaterals on the abdominal wall and edema in the lower extremities may suggest the presence of IVC obstruction.

Membranous obstruction of the IVC is most commonly encountered in young adults in the Far East and Africa. Its course is subacute, in contrast to the usually acute presentation of thrombotic occlusion of the IVC.

Angiography demonstrates a filling defect in the IVC below the level of the diaphragm or a web with a collateral venous pattern. The hepatic veins are usually patent, with a high WHVP but a normal hepatic venous pressure gradient (HVPG) resulting from high IVC pressure. Patients with an IVC web may be cured by surgical excision or percutaneous angioplasty.

Heart disease

When pressure rises in the right chambers of the heart in constrictive pericarditis, valvular disease (e.g., mitral stenosis with tricuspid insufficiency), or cardiomyopathy of any cause, the pressure is transmitted backward through a valveless venous system from the IVC to the hepatic veins, hepatic sinusoids, and portal venous system. The consequence is a picture similar to that of hepatic vein occlusion, IVC web, or any syndrome of obstruction of the hepatic venous outflow. The symptoms of such patients—intractable ascites and hepatocellular dysfunction—may be difficult to distinguish from those associated with other causes of portal hypertension, even cirrhosis. Esophageal varices are usually not present because a gradient between the portal and azygos systems is lacking.

Pathophysiology of Portal Hypertension

PPG is the result of the interaction between the portal blood flow and the vascular resistance to the flow. This relationship is defined by Ohm's law in the following equation:

$\Delta P = Q \times R$

in which ΔP is the PPG (the difference between the portal pressure and the IVC pressure), Q is the blood flow within the entire portal venous system (which in portal hypertension includes the portal–systemic collaterals), and R is the vascular resistance of the entire portal venous system. It follows that portal pressure may be increased by an increase in portal blood flow, an increase in vascular resistance, or a combination of both (23). It is well established that in cirrhosis, the primary factor leading to portal hypertension is an increased resistance to portal blood flow. An increased portal venous inflow maintains and exacerbates portal hypertension. This component of increased blood flow becomes especially important in advanced stages (Fig. 15.1).

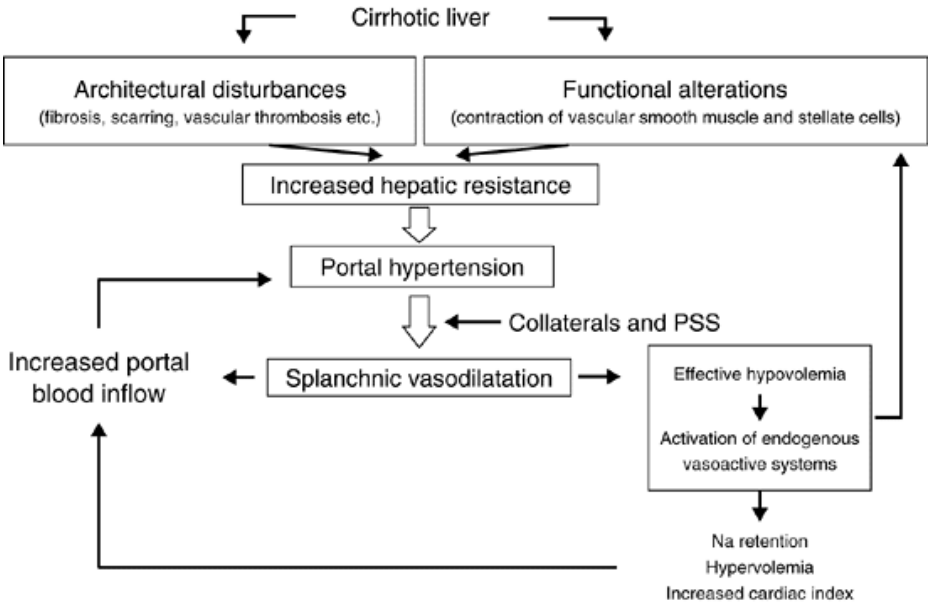
Increased Vascular Resistance to Portal Blood Flow

Increased resistance to portal blood flow is the primary factor in the pathophysiology of portal hypertension and may occur at any site within the portal venous system. In cirrhosis, increased intrahepatic vascular resistance is thought to be mainly in the hepatic sinusoids (5). For many years, the increased intrahepatic vascular resistance was thought to be a fixed, mechanical consequence of architectural distortion of the hepatic microcirculation by fibrosis, scarring, and nodules. In addition, careful pathologic studies have suggested that thrombosis of medium and large portal and hepatic veins is a frequent occurrence in cirrhosis and that these events may be important in causing a progression of cirrhosis and worsening of portal hypertension (24).

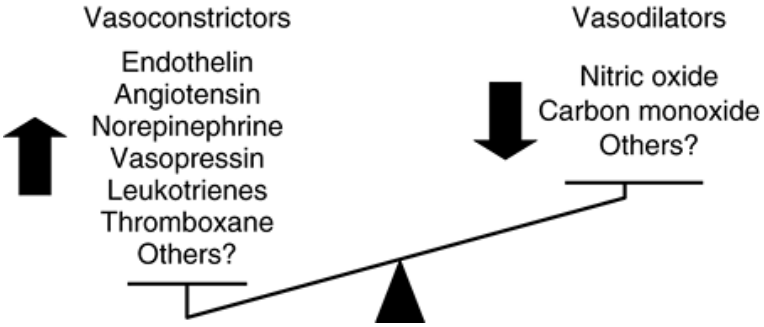
However, recent studies have demonstrated that in addition to the increased resistance caused by the morphologic changes of chronic liver diseases, a dynamic component of increased resistance is present that represents active contraction of the contractile elements in the liver. These elements constrict in a reversible and graded manner in response to several agonists, thereby further increasing the intrahepatic resistance (25). It has been claimed that this dynamic component may represent up to 40% of the increase in intrahepatic vascular resistance.

The contractile elements of the hepatic vascular bed are located at a sinusoidal level and at extrasinusoidal sites (26,27,28). They include smooth muscle cells of the intrahepatic vasculature (i.e., small portal venules in

portal areas) (26); activated hepatic stellate cells, which are located in the perisinusoidal space of Disse and have extensions that wrap around the sinusoids (27); and hepatic myofibroblasts, which are abundant in the fibrous tissue in and around cirrhotic nodules. Contraction of hepatic myofibroblasts may increase intrahepatic resistance by compressing venous shunts in the fibrous septa. It is now clear that vasoactive mediators, either vasoconstrictors or vasodilators, modulate intrahepatic vascular resistance in both the healthy and the cirrhotic liver. An increased production of vasoconstrictors and an exaggerated response of the hepatic vascular bed to these agents, as well as an insufficient release of vasodilators, together with an impaired vasodilatory response of the hepatic vascular bed, are the mechanisms that have been implicated in the pathogenesis of the dynamic component of the increased intrahepatic resistance of the cirrhotic liver (5) (Fig. 15.2).



▪ **Figure 15.1** Schematic representation of the pathophysiology of portal hypertension.



▪ **Figure 15.2** Factors modulating intrahepatic resistance in cirrhosis. A reduction in the release of endogenous vasodilators and an increase in vasoconstrictor activity contribute to the increased hepatic vascular resistance in cirrhosis.

Increased Production of Vasoconstrictors and Exaggerated Response of the Hepatic Vascular Bed

Endothelins (*ETs*) are a family of homologous 21 amino acid vasoactive peptides (ET-1, ET-2, and ET-3) that are thought to play a major role in modulating hepatic vascular tone in cirrhosis (29). The biologic properties of ETs are mediated essentially by two major ET receptors, ET-A and ET-B. The ET-A receptor shows a high affinity for ET-1, but not for ET-3, and mediates constriction; the ET-B receptor has equal affinity for ET-1 and ET-3. Activation of ET-B receptors located on the vascular smooth muscle cells promotes vasoconstriction, whereas activation of ET-B receptors located on

endothelial cells promotes vasodilatation, which is mediated by enhanced nitric oxide (NO) and prostacyclin production by the endothelial cell.

Patients with liver cirrhosis have increased circulating plasma levels of ET-1 and ET-3 (30). The increase is greater and more consistently found in patients with cirrhosis and ascites. A net release of ET-1 and ET-3 during splanchnic passage has been observed in patients with cirrhosis, but not in controls, a finding that suggests an increased production of ET-1 and ET-3 in the splanchnic territory. Immunostaining and in situ hybridization studies have detected an increased expression of ET-1 in human cirrhotic livers (31); endothelial cells, hepatic stellate cells (in their activated phenotype), and bile duct epithelial cells are the major intrahepatic sources of ET-1. A decreased clearance of ET-1 by the liver has also been demonstrated in cirrhotic rats.

ET-1 increases portal perfusion pressure by increasing intrahepatic resistance in isolated, perfused normal livers and carbon tetrachloride–induced cirrhotic livers. Although some experimental studies reported a slight reduction of portal pressure in cirrhotic animals after the administration of ET antagonists (32,33), this was not confirmed by other studies (34). Therefore, the role of ETs in increasing the vascular tone in cirrhosis remains unsettled.

Other vasoconstrictive factors are involved in the regulation of hepatic vascular tone. Studies in perfused cirrhotic livers have shown that *norepinephrine, angiotensin II*, and *vasopressin*, three circulating vasoactive factors whose levels are usually elevated in cirrhosis, increase intrahepatic vascular resistance (35,36). The increased resistance promoted by norepinephrine is completely blunted by the administration of α-adrenergic antagonists, such as prazosin. These agents by themselves markedly reduce hepatic resistance and portal pressure in patients with cirrhosis. On the other hand, the administration of α-adrenergic agonists, such as isoproterenol, reduces intrahepatic vascular resistance in perfused cirrhotic liver. These data suggest that adrenergic receptors may be involved in the regulation of intrahepatic resistance in cirrhosis and that α-adrenergic receptor blockers may decrease portal pressure in cirrhosis. In addition, the hepatic vascular bed of cirrhotic livers exhibits an exaggerated response to the α-adrenergic agonist methoxamine. This hyper-response is associated with the overproduction of thromboxane A_2 (TXA_2) by cyclo-oxygenase (COX-1) isoenzyme and is completely corrected by pretreating the livers with nonselective COX blockers, COX-1–selective blockers, or TXA_2 antagonists. Therefore, an increased production of TXA_2 markedly enhances the vasoconstrictive response of the cirrhotic hepatic vascular bed to methoxamine (37). Whether this effect is also shared by other vasoconstrictors has not been investigated so far. However, it is known that the coupling of different agonists to the membrane G-coupled receptors promotes the release of arachidonic acid from the plasma membrane, facilitating its metabolization to different prostanoids (38,39).

Angiotensin II is a powerful vasoconstrictor that may increase hepatic resistance. Strategies based on direct angiotensin II blockade by specific A-II antagonists, inhibitors of the converting enzyme, or A-II receptors blockers may reduce portal pressure but cause systemic hypotension.

Cysteinyl leukotrienes are a group of highly potent vasoactive substances derived from the oxygenation and dehydration of arachidonic acid by 5-lipoxygenase. These substances have been shown to increase intrahepatic vascular resistance in normal and carbon tetrachloride–induced cirrhotic rat livers. The response is significantly greater in cirrhotic livers, which exhibit increased production of cysteinyl leukotriene and expression of 5-lipoxygenase messenger ribonucleic acid (mRNA). In addition, inhibition of 5-lipoxygenase produces a significant and marked reduction in portal pressure in cirrhotic livers, which suggests that 5-lipoxygenase–derived eicosanoids contribute to the increased hepatic vascular resistance in cirrhosis.

Endothelial Dysfunction of Cirrhotic Livers

In normal conditions, the endothelium is able to generate vasodilator stimuli in response to increases in blood volume, blood pressure, or vasoconstrictor agents in an attempt to prevent or attenuate the concomitant increase in pressure. In several pathologic conditions there is an impairment in this endothelium-dependent vasodilatation, called *endothelial dysfunction* (40,41). Endothelial dysfunction is considered one of the main pathologic mechanisms involved in the increased vascular tone observed in several vascular disorders, such as arterial hypertension, diabetes, and atherosclerosis, and has been attributed to a diminished NO bioavailability (40,41) or to an increased production of endothelial-derived contracting factors, such as prostaglandin H_2 (PGH_2)/TXA_2 (42), ET (43), or anion superoxide (44).

The hepatic vascular bed of cirrhotic livers also exhibits endothelial dysfunction (45). Indeed, studies performed both in patients with cirrhosis and in experimental models have shown that, contrary to what happens in normal livers, the cirrhotic liver cannot accommodate the increased portal blood flow caused by the postprandial hyperemia, which determines an abrupt postprandial increase in portal pressure (46). This is important because such repeated brisk increases in portal pressure and portal–collateral blood flow in response to meals and other physiologic stimuli are thought to be a major determinant of the progressive dilatation of the varices in patients with cirrhosis (5). In addition, endothelial dysfunction has been further characterized in experimental models of cirrhosis by showing that the cirrhotic liver exhibits an impaired response to the endothelium-dependent vasodilator acetylcholine (45,47). This impaired response to acetylcholine was shown to be associated with an increased production of TXA_2 and completely prevented by selective COX-1 blockers and TXA_2 antagonists. These results suggest that an increased production of a COX-1–derived vasoconstrictor prostanoids, probably TXA_2, is, at least in part, responsible for endothelial dysfunction (47). Acetylcholine coupling to the endothelial muscarinic M3 receptor has been shown to promote the stimulation of NO synthase and COX-1, with the subsequent release of NO and vasoconstrictor endoperoxides, respectively (48). In physiologic states,

a vasodilating response is the final balance between the interactions of these endothelial vasoactive mediators. However, in cirrhosis, as in other conditions such as hypertension, diabetes, and arteriosclerosis, there is a perturbation of this balance, resulting in endothelial dysfunction with the consequent impaired response to acetylcholine. All these findings suggest that in cirrhotic livers there is an overactivation of the COX-1 pathway with an increased production of the vasoconstrictor-derived compounds.

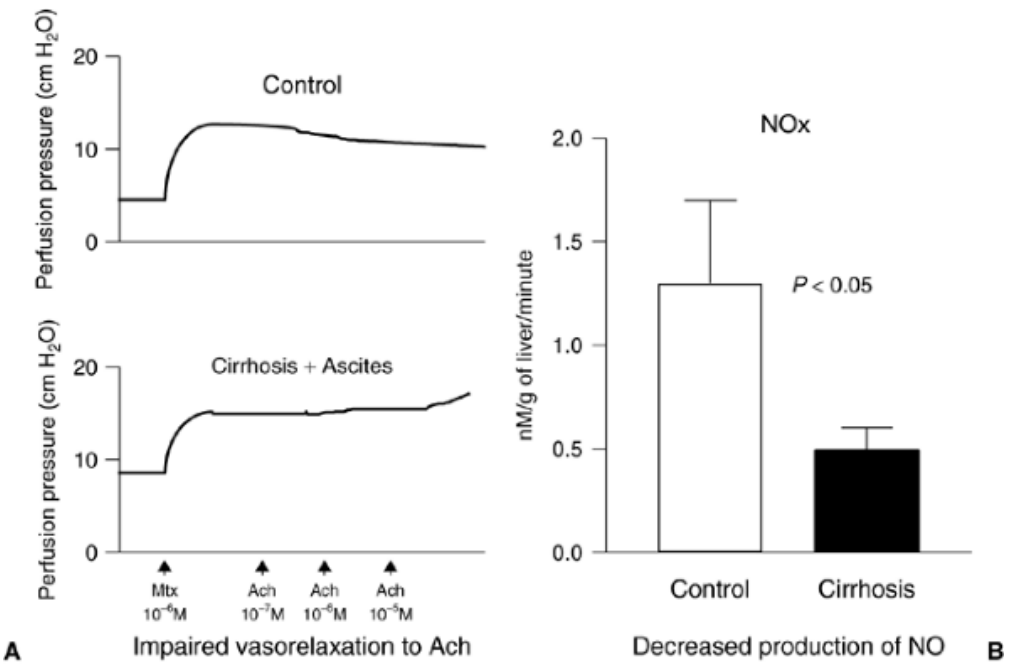
Insufficient Release of Hepatic Vasodilators

Nitric oxide

The role of NO in modulating intrahepatic vascular resistance is a subject of considerable interest. NO is a powerful endogenous vasodilator generated in several tissues by NO synthases from the amino acid L-arginine. It is the natural ligand for soluble guanylate cyclase and is responsible for an increase in the levels of cyclic guanosine monophosphate, the final agent responsible for the relaxation of the vascular wall through the extrusion of cytosolic Ca^{2+}.

NO blockade has been shown to increase portal perfusion pressure in isolated perfused rat livers. In addition, the hepatic response to norepinephrine is markedly enhanced after NO inhibition, a finding that further suggests a role for NO in modulating hepatic vascular tone in normal conditions (49). In the cirrhotic liver, the synthesis of NO is insufficient to compensate for the activation of vasoconstrictor systems frequently associated with cirrhosis (Fig. 15.3). This occurs despite a normal expression of

endothelial nitric oxide synthase (eNOS) mRNA and normal levels of eNOS protein (45,50). The decreased activity of hepatic eNOS in cirrhosis is due in part to increased expression of caveolin (51). This insufficient hepatic NO generation plays a major role in increasing intrahepatic vascular resistance in cirrhosis, thereby worsening portal hypertension. In accordance with this concept, the infusion of L-arginine, the precursor of NO biosynthesis, and the administration of nitrates (exogenous donors of NO) have been shown to decrease portal pressure. In addition, enhancement of the expression of NO synthase in liver cells, through the portal injection of adenovirus coupled with the gene encoding NO synthase, significantly reduces portal pressure (52). More recently, strategies aimed at increasing NO release by enhancing intrahepatic eNOS activity, on the basis of constitutively active *Akt* gene transfer (53), or by simvastatin administration (54) have opened new perspectives with potential therapeutic implications.



▪ **Figure 15.3** Cirrhotic rat livers exhibit endothelial dysfunction, characterized by an impaired vascular relaxation in response to acetylcholine (Ach) **(A)** and a decreased production of nitric oxide (NO) **(B)**. NOx, nitrite/nitrate; Mtx, methotrexate. (From Gupta TK, Tourner M, Chung MK, et al. Endothelial dysfunction and decreased production of nitric oxide in the intrahepatic microcirculation of cirrhotic rats. *Hepatology* 1998;28:926–931, with permission.)

Carbon monoxide

A role for carbon monoxide (CO), a by-product of heme group oxidation by heme oxygenases (HOs), as an important modulator of intrahepatic vascular tone has been suggested. CO, although less potent than NO, also activates guanylate cyclase and thereby

promotes smooth muscle relaxation. The inhibition of CO production increases portal resistance in normal livers (55).

Heme oxidation is catalyzed by two different enzymes, HO-1 and HO-2. In normal conditions, CO is produced in the liver by the constitutive isoform (or HO-2). However,

in several stress conditions, such as endotoxemia and hemorrhagic shock, the inducible isoform (or HO-1) is formed. In this situation, the inhibition of HOs with specific agents leads to a much greater increase in portal–hepatic resistance than is seen in normal, unstimulated livers (56).

HO-1 expression is increased in the liver in experimental models of cirrhosis (57). In addition, there is a complex interaction between the NO and CO systems. NO has been shown to induce HO-1 expression and, therefore, CO synthesis. On the other hand, CO may inhibit the NO-mediated production of cyclic guanylate cyclase monophosphate. Therefore, an increased expression of HO-1 in cirrhosis suggests that CO may play a role in the hepatic circulatory disturbances found in liver cirrhosis.

Splanchnic Vasodilatation

An increased portal venous inflow is characteristically observed in advanced stages of portal hypertension and is the result of marked arteriolar dilatation in the splanchnic organs draining into the portal vein. The increased blood flow contributes to the portal hypertensive syndrome (5) (Fig. 15.1).

Different mechanisms have been suggested to explain the observed hemodynamic abnormality, which likely represents a multifactorial phenomenon involving neurogenic, humoral, and local mechanisms. Initial studies focused on the potential role of increased levels of circulating vasodilators. Many candidate substances were proposed, most of them being vasodilators of splanchnic origin that undergo hepatic metabolism and accumulate in the systemic circulation when hepatic uptake is reduced in liver disease or during portosystemic shunting.

Glucagon

Glucagon is probably the humoral vasodilator for which most evidence has been accumulated to indicate a significant role for it in splanchnic hyperemia and portal hypertension.

Many studies have demonstrated that plasma glucagon levels are elevated in patients with cirrhosis and experimental models of portal hypertension. Hyperglucagonemia results, in part, from a decreased hepatic clearance of glucagon, but more importantly from an increased secretion of glucagon by pancreatic α cells (58). The support for a role of glucagon in modulating splanchnic blood flow comes from physiologic studies showing that in rats with experimental portal hypertension, normalizing circulating glucagon levels by administering glucagon antibodies or infusing somatostatin partially reverses the increase in splanchnic blood flow, a response that can be specifically blocked by the concomitant infusion of glucagon (59,60). Conversely, other studies have shown that increasing circulating glucagon levels in normal rats to values similar to those observed in portal hypertension causes a significant increase in splanchnic blood flow. On the basis of these studies, it has been suggested that hyperglucagonemia may account for approximately 30% to 40% of the splanchnic vasodilatation of chronic portal hypertension. Glucagon may promote vasodilatation by a dual mechanism: Relaxing the vascular smooth muscle and decreasing its sensitivity to endogenous vasoconstrictors, such as norepinephrine, angiotensin II, and vasopressin (61,62). The role of glucagon in the splanchnic hyperemia of portal hypertension provides a rationale for the use of somatostatin and its synthetic analogs to treat portal hypertension (63).

Endocannabinoids

Recent data suggest a role for endocannabinoids in the hyperdynamic circulation of portal hypertension (64,65). Increased levels of the endogenous cannabinoid anandamide have been found in the monocyte fraction of blood from cirrhotic humans and rats, and an increased expression of the cannabinoid CB1 receptors was found in

hepatic human endothelial cells (64). In addition, CB1 receptor blockade has been found to reduce portal blood flow and pressure and increase arterial pressure in cirrhotic rats (64,65). The mechanism of action is not well understood. It has been suggested that it could be due, at least in part, to an increased NO production, mediated by the activation of endothelial CB1 receptors (64). However, the data are not conclusive (65).

Several other circulating vasodilators have been implicated in splanchnic vasodilatation. *Bile acids* are increased in portal hypertension and have vasodilator properties. However, the data in the literature are controversial, and the role of bile acids in the hyperdynamic circulation is not well defined. Likewise, the role of the *capsaicin–calcitonin gene-related peptide* vasodilator pathway in the systemic and splanchnic vasodilatation of portal hypertension is controversial. Other candidates, including neuropeptides, adenosine, endotoxin, and a variety of vasodilator gastrointestinal hormones, have also been studied. However, supporting evidence is scarce for most of them.

Nitric oxide

Experimental studies of specific NO inhibitors have shown that NO is involved in the regulation of

splanchnic and systemic hemodynamics in portal hypertensive and control animals. The splanchnic vasoconstrictive effect caused by NO inhibitors is significantly greater in portal hypertensive than in control animals, which suggests that an excessive production of NO may be responsible, at least in part, for the vasodilatation observed in portal hypertension (66,67). In addition, NO inhibition has been shown to reverse the vascular hyporesponsiveness to vasoconstrictors that is characteristic of portal hypertension and is thought to contribute to systemic and splanchnic vasodilatation (68). In addition, an overproduction of NO has been clearly demonstrated in vitro in perfused mesenteric artery preparations from portal hypertensive rats (69). The finding in patients with cirrhosis of increased serum and urinary concentrations of nitrite and nitrate, which are products of NO oxidation, also supports a role for NO in the genesis of the circulatory disturbances of portal hypertension (70).

The increased production of NO is due both to an increased expression and an increased activity of eNOS (71,72). Factors likely to activate the constitutive NO synthase include shear stress, circulating vasoactive factors (e.g., ET, angiotensin II, vasopressin, and norepinephrine) and overexpression of the angiogenic factor vascular endothelial cell growth factor (VEGF) (73,74). In portal hypertensive animals, NO overproduction by eNOS in the splanchnic circulation precedes the development of hyperdynamic circulation (75) and is mostly due to an upregulation of eNOS catalytic activity, rather than eNOS overexpression (76). Indeed, eNOS phosphorylation by Akt seems to be the mechanism of the initial upregulation of eNOS activity and NO-mediated hyporesponsiveness to vasoconstrictors (76). Later on, other mechanisms become important, including an enhanced signaling of the molecular chaperone heat shock protein 90 (Hsp90) (77,78). In addition, in cirrhotic rats with ascites, it was shown that bacterial translocation further increased eNOS expression in the mesenteric artery, probably in response to increased levels of tumor necrosis factor α (TNF-α). TNF-α production was associated with elevated levels of tetrahydrobiopterin, a TNF-α–stimulated cofactor and enhancer of eNOS-derived NO biosynthesis and NO synthase activity (72).

Prostaglandins

Several studies support a role for prostaglandins in the hyperdynamic circulation in portal hypertension (79,80,81). Prostacyclin is an endogenous vasodilator produced by vascular endothelial cells. It causes vascular smooth muscle relaxation by activating

adenylate cyclase and augmenting the intracellular level of cyclic adenosine monophosphate. Two different isoforms of COX are involved in the biosynthesis of prostacyclin. The constitutive isoform (COX-1) may be stimulated by factors similar to those that stimulate the constitutive isoform of NO synthase (eNOS). In addition, the inducible isoform (COX-2), like inducible nitric oxide synthase, can be expressed on stimulation with proinflammatory agents. Animal studies have shown a partial reversal of splanchnic vasodilatation after COX blockade. This effect is independent of that of NO. They have further shown that systemic levels of prostacyclin may be increased in patients with cirrhosis. In addition, the inhibition of prostaglandin biosynthesis by indomethacin reduces the hyperdynamic circulation and portal pressure in patients with cirrhosis and portal hypertension. Recent studies suggest that both isoenzymes COX-1 and COX-2 are involved in the increased prostacyclin production by the mesenteric vascular bed of portal vein–ligated rats (82). The selective inhibition of COX-2, and to a lesser extent of COX-1, improved the endothelial-dependent vasodilatation in response to acetylcholine (82).

Carbon monoxide

Recent studies have shown an increased expression and activity of the inducible form, or HO-1, in splanchnic tissues from animals with portal hypertension (57,83). In addition, the simultaneous inhibition of NO and HO has been shown to completely reverse the reduced vasoconstrictor response to potassium chloride in the mesenteric vascular bed (83).

Altogether, these data suggest that the splanchnic vasodilatation present in portal hypertension is likely to be multifactorial in origin, being promoted in part by an excessive release of NO, CO, and other vasoactive mediators. In addition, experimental studies suggest that when one of the vasoactive mediators is chronically inhibited, the enhancement of other vasoactive pathways may prevent the correction of splanchnic vasodilatation (84). Coupling of several vasoactive systems may cause the splanchnic vasodilatation seen in portal hypertensive states.

Portosystemic Collateral Circulation

The development of portal–collateral circulation is one of the main complications of portal hypertension. Formation of collaterals is a complex process involving the opening, dilatation, and hypertrophy of preexisting vascular channels (4). Collaterals develop in response to the increased portal pressure. A minimum HVPG threshold of 10 mm Hg should be reached for the development of portosystemic collaterals and esophageal varices (2,85). In addition to the increased portal pressure, recent studies have shown that formation

of portosystemic collateral vessels in portal hypertension is influenced by a VEGF-dependent angiogenic process and can be markedly attenuated by interfering with the VEGF/VEGF receptor-2 signaling pathway (73,74). These studies have opened a new perspective in the understanding of the pathophysiology of portal hypertension, with potential clinical relevance, because these studies indicate that manipulation of the VEGF may be of therapeutic value.

The collateral circulation may carry as much as 90% of the blood entering the portal system. In this circumstance, the vascular resistance of these vessels becomes a major component of the overall resistance to portal blood flow and, therefore, may be important in determining portal pressure. In addition, although it was traditionally thought that the hyperdynamic splanchnic circulatory state associated with portal hypertension was the consequence of active splanchnic vasodilatation, recent data suggests that the increased neovascularization in splanchnic organs plays an important role in allowing the increase in splanchnic blood inflow (74).

The elements that modulate collateral resistance are not well known. Studies

performed in perfused portosystemic collateral beds suggest that NO may play a role in the control of portal collateral vascular resistance (86). This may be the mechanism by which isosorbide-5-mononitrate (IMN) and nitroglycerin (NTG) reduce collateral resistance in patients with cirrhosis. These vessels are also probably hypersensitive to serotonin (5-HT), which markedly increases their vascular tone. In portal hypertensive animals, the administration of selective 5-HT2 receptor blockers decreases portal pressure.

Vasoconstrictive agents (including vasopressin and nonselective β-blockers) may significantly increase the collateral resistance. The increase in portal collateral resistance brought about by these agents attenuates the reduction in portal pressure achieved by reducing the splanchnic blood flow (87). Another circumstance in which active changes in portal collateral resistance appear to modulate changes in portal pressure is the restitution of blood volume after hemorrhage, during which a paradoxical increase in portal pressure occurs in portal hypertensive animals.

Increased Plasma Volume and Hyperkinetic Circulation

Splanchnic vasodilatation is typically associated with peripheral vasodilatation and a systemic hyperkinetic syndrome, which is characterized by reduced arterial pressure and peripheral resistance, and increased plasma volume and cardiac output. The pathophysiologic mechanisms involved in peripheral vasodilatation are similar to those previously described for splanchnic vasodilatation (5). Peripheral vasodilatation plays a major role in the activation of endogenous neurohumoral systems that cause sodium retention and expansion of the plasma volume, followed by an increase in the cardiac index (88). The relevance of these abnormalities to the pathophysiology of ascites and the hepatorenal syndrome is discussed in detail in Chapters 7 and 9.

Expansion of plasma volume is a necessary step to maintain an increased cardiac index, which in turn aggravates portal hypertension (88). This provides the rationale for using a low-sodium diet and diuretics in the treatment of portal hypertension.

Pathophysiology of Variceal Hemorrhage

Two theories have been proposed to explain variceal bleeding (Fig. 15.4). The *erosion theory* suggested that varices bleed when external trauma to their thin and fragile walls is caused by the deglutition of solid food or by gastroesophageal reflux. This theory has been abandoned because of a lack of objective evidence. No relationship between eating and bleeding has been proved, nor is the incidence of reflux and esophagitis greater in patients with bleeding varices than in those without bleeding (5,89).

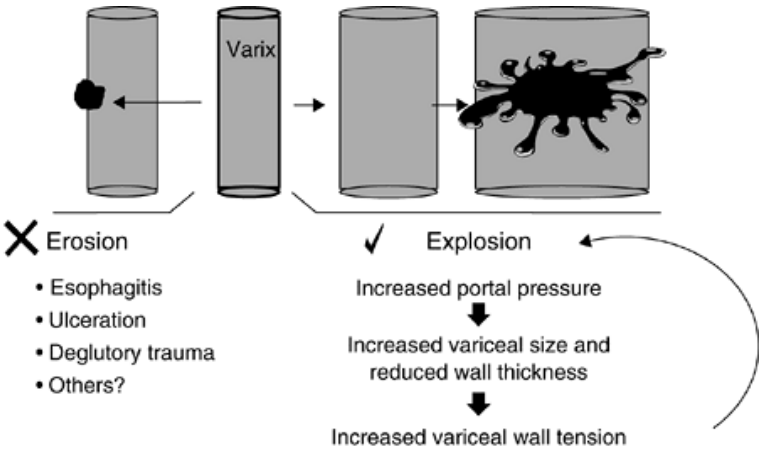
On the contrary, the so-called explosion hypothesis suggests that the main cause of bleeding is excessive hydrostatic pressure inside the varices, which is a consequence of increased portal pressure (Fig. 15.4). In support of this hypothesis, many studies have shown that variceal bleeding does not occur before the HVPG reaches a threshold value of 12 mm Hg (1,3,4) (Fig. 15.5). In addition, since the introduction of endoscopic techniques to measure variceal pressure (see subsequent text), new observations have been made to support the role of increased intravariceal pressure in variceal rupture. Therefore, variceal pressure is higher in patients with previous bleeding than in nonbleeders, and longitudinal studies have shown that variceal pressure is a good prognostic indicator of the risk for bleeding and of the response to pharmacologic therapy.

Variceal pressure, size, and wall thickness can be integrated in the concept of *wall tension*, the inwardly directed force exerted by the variceal wall to oppose an outwardly directed force that causes further distention (Fig. 15.6). Variceal bleeding occurs when the tension exerted by the thin wall of a varix is beyond a critical value, as determined by the elastic limit of the vessel. At this point, the variceal wall cannot

resist further dilatation, and variceal rupture occurs. According to Frank's modification of Laplace's law, variceal wall tension (*WT*) can be defined by the following equation:

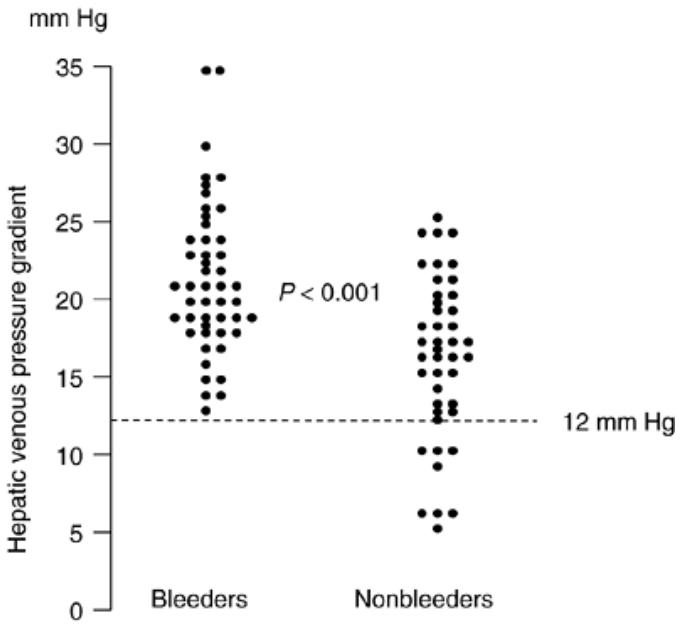
$WT = (P_i - P_e) \times r/w$

in which P_i is the intravariceal pressure, P_e the pressure in the esophageal lumen, *r* the radius of the varix, and *w* the thickness of its wall (5,89).



▪ **Figure 15.4** Proposed theories of the mechanism of variceal hemorrhage. Variceal bleeding occurs when the thin wall of a varix leaks. The erosion theory proposes that leaks are caused by external injury. On the contrary, today's accepted "explosion" theory proposes that variceal rupture is mainly a consequence of increased hydrostatic pressure inside a varix, which causes an increase in the tension of the variceal wall to above its elastic limit.

This equation indicates that a large variceal size exaggerates the deleterious effects of a high intravariceal pressure, so that the tension exerted on the wall of the varix is increased; at a much lower variceal pressure the wall tension (and risk for bleeding) of a big varix with thin walls will be higher than that of a small varix with thick walls. This may explain why large gastric fundal varices bleed at relatively low portal pressures. Similarly, the equation explains the prognostic value of the red color signs (which indicate areas where the wall of a varix is especially thin) (90,91).



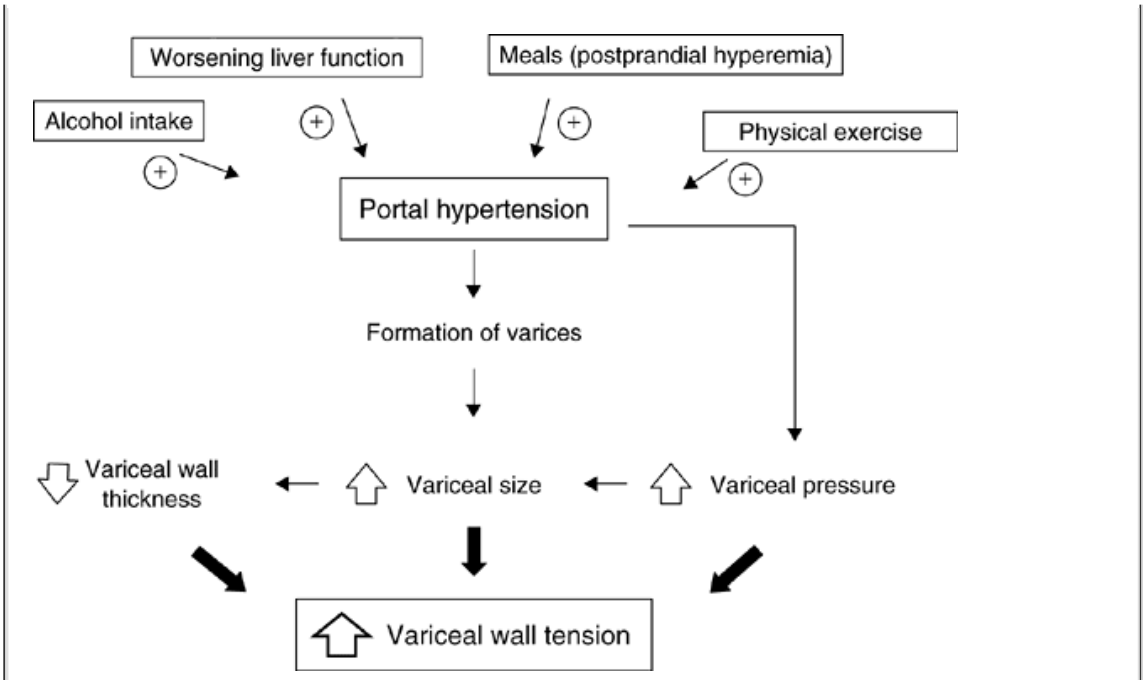
▪ **Figure 15.5** Variceal bleeding occurs only in patients with a hepatic venous pressure gradient above 12 mm Hg (the threshold value for variceal bleeding). (From Garcia-Tsao G, Groszmann RJ, Fisher RL, et al. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 1985;5:419–424, with permission.)

The concept of variceal wall tension explains why esophageal varices are more likely to bleed than other collaterals, in the thorax (e.g., the periesophageal veins), gut, or other abdominal organs (ectopic varices). The transmural pressure is higher in esophageal varices than in varices at other locations because negative esophageal luminal pressure occurs during inspiration. Furthermore, the lack of external tissue support of esophageal varices decreases their elastic limit. The factors determining variceal wall tension are interrelated. Increased variceal pressure increases wall tension directly; it also increases the size (radius) of a varix and, by the same mechanism, decreases wall thickness (Fig. 15.6).

Accordingly, the natural history of portal hypertension can be described as a function of variceal wall tension. Once wall tension increases to values exceeding the elastic limit of a varix, the patient experiences a first episode of bleeding. After this, the patient remains at a high risk for rebleeding unless wall tension is decreased. Similarly, primary prophylaxis protects the patient from bleeding by preventing or delaying variceal wall tension from reaching the rupture point, which is achieved by decreasing portal pressure and portal–collateral blood flow.

The sequence of events leading to variceal hemorrhage is therefore initiated by a high portal pressure, which promotes the formation of collaterals and varices. An increase in intravascular pressure, together

with a high rate of collateral blood flow, causes varices to dilate, and as they dilate, their walls become thinner (Fig. 15.6). At this point, any further increase in variceal pressure or size or any defect in the variceal wall causes rupture and clinical hemorrhage.



▪ **Figure 15.6** The factors determining variceal wall tension are interrelated. An increase in variceal pressure increases wall tension directly; it also increases the size (radius) of the varix and decreases the thickness of its wall. As indicated, repeated increases in portal pressure caused by several factors contribute to accelerate this process.

In this regard, it should be remembered that portal pressure and blood flow are not static parameters; on the contrary, they vary markedly in response to physiologic stimuli. It is well known that portal pressure increases transiently after a meal in relation to the postprandial hyperemic response. Alcohol intake, physical exercise, and conditions that increase intra-abdominal pressure can increase portal pressure abruptly (Fig. 15.6). In all these circumstances, repeated abrupt increases in portal pressure cause a progressive dilatation of varices and, therefore, increase the risk for variceal bleeding. These observations are relevant because they suggest important new therapeutic targets to reduce the risk for variceal hemorrhage. Moreover, circadian variations have been observed in portal pressure—pressure increases during the night and decreases during the afternoon and evening. These physiologic variations in portal pressure may affect the onset of bleeding in patients at risk (those with a high variceal tension in resting conditions); a circadian pattern has been observed in variceal hemorrhage, which is more frequent at midnight, when portal pressure generally is increasing. In patients with cirrhosis, portal pressure is also increased by circumstances that worsen liver failure, such as alcoholic hepatitis, severe infections, and acute or chronic liver failure.

Assessment of Hepatic Hemodynamics in Patients with Cirrhosis

The evaluation of patients with portal hypertension is based on the visualization of varices at endoscopy, definition of the portal collateral anatomy by ultrasonography or angiography, and the measurement of portal pressure. There are also techniques to assess changes in pressure and blood flow in the collateral circulation. These have allowed a better understanding of the hemodynamic changes associated with portal hypertension, the mechanism of bleeding, and the effects of new forms of

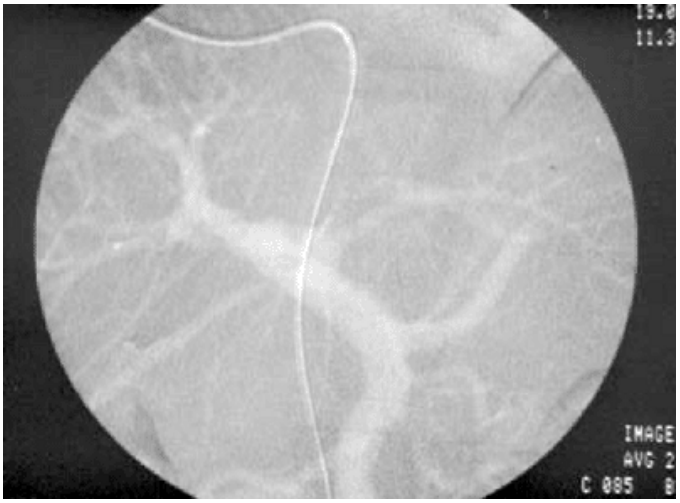
pharmacologic therapy.

Imaging Techniques: Portal Venography, Ultrasonography, Pulsed Doppler Ultrasonography, Computed Tomography, and Magnetic Resonance Imaging

Imaging techniques are useful in the initial evaluation of the patient with portal hypertension. Frequently, portal hypertension is first diagnosed when a dilated portal vein, portosystemic collaterals, ascites, or splenomegaly is detected.

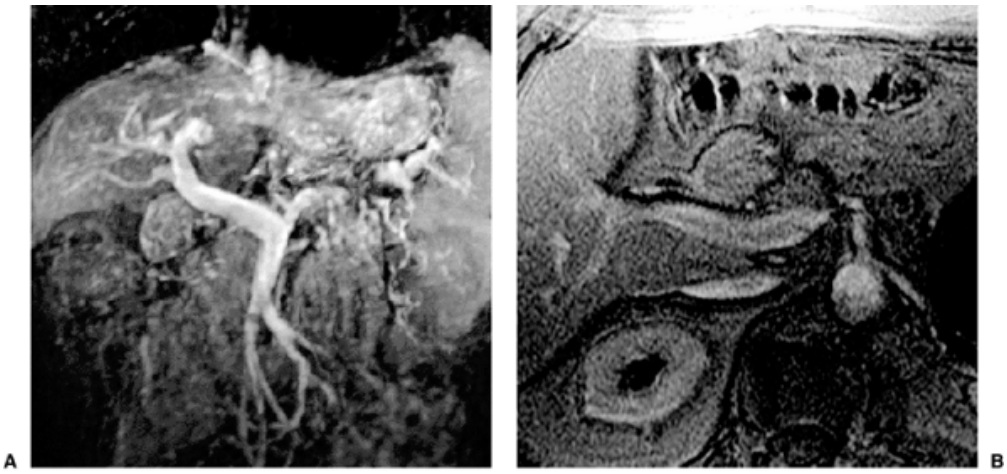
The patency of the portal vein or the presence of PVT should be investigated in every patient with portal hypertension. Portal venography obtained at the venous phase of splenic and mesenteric angiography is being

replaced by noninvasive methods, such as Doppler ultrasonography with echo enhancement, helical CT scan, and MRI. Retrograde wedged hepatic venography, performed with the use of carbon dioxide as a contrast medium during hepatic vein catheterization, is a safe technique that allows an adequate visualization of the portal vein in more than 70% of patients with cirrhosis (92) (Fig. 15.7).



▪ **Figure 15.7** Wedged retrograde portography with the use of carbon dioxide as a contrast agent shows patent portal, mesenteric, and splenic veins and extensive collateralization in a patient with hepatitis C–related cirrhosis.

Ultrasonography, CT scan, and MRI are as accurate as angiography in detecting PVT (Fig. 15.8). Ultrasonography is the preferred initial investigation because of its low cost and high accuracy (93,94,95). The use of pulsed Doppler (the so-called duplex technique) allows the determination of the presence, direction, and velocity of portal blood flow. Multiplying the portal flow velocity by the cross-sectional area of the portal vein provides an estimate of the portal blood flow. However, these estimates are subject to many errors and should be used with caution. Ultrasonography and pulsed Doppler flow measurements are also useful in the assessment of the patency of transjugular intrahepatic or surgical portacaval shunts and in the evaluation of the patency of arterial and venous anastomoses after orthotopic liver transplantation.



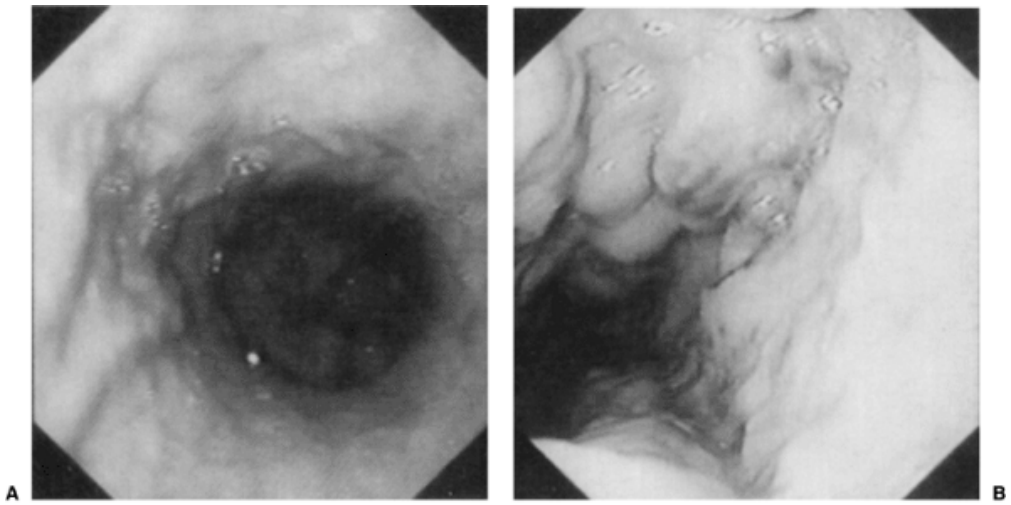
▪ **Figure 15.8** Magnetic resonance image shows a patent portal vein (sagittal and coaxial sections). This technique also allows an estimation of portal blood flow. (Courtesy of Dr. J. R. Ayuso.)

Promising results have been obtained by measuring portal blood flow with phase-contrast magnetic resonance angiography, although the clinical applications of this method are still under study.

Endoscopy

As discussed later in this chapter (see "Natural History and Clinical Manifestations of Portal Hypertension"), upper gastrointestinal endoscopy is mandatory in patients with cirrhosis or in those in whom portal hypertension is suspected. At endoscopy, it is important to semiquantitatively assess the number, appearance, and size of any esophageal varix and to note the presence of red color signs (Fig. 15.9). Endoscopy should include a careful evaluation for the presence of gastric varices (Fig. 15.10), which in some cases may require endosonography, and the presence, extent, and severity of PHG (Fig. 15.11). It can be useful to standardize the moment at which the varices are observed (i.e., at full inflation, before removal of the endoscope) and obtain digital images for later review, especially given the fact that these patients require follow-up endoscopy. It is

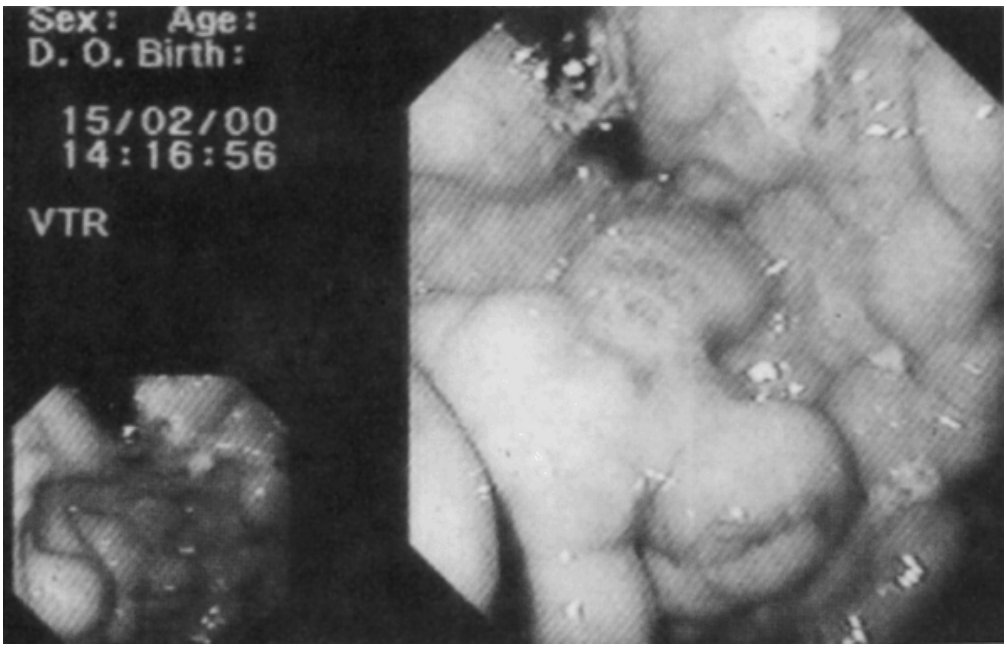
also important to provide conscious sedation because it markedly increases the acceptability of the procedure. Capsule endoscopy is now available for the examination of the upper gastrointestinal tract; initial studies suggest a high sensitivity in the detection of esophageal varices.



▪ **Figure 15.9** Esophageal varices. Small varices **(A)** and large esophageal varices with red color signs **(B)**. (Courtesy of Drs. J. M. Bordas and J. Llach.)

Measurement of Portal Pressure

Measurement of portal pressure is still the single most important hemodynamic measurement in portal hypertension. Other methods provide additional information but do not substitute for the measurement of portal pressure. Portal pressure should be expressed in terms of the pressure gradient between the porta and the IVC, which represents the perfusion pressure within the portal and hepatic circulation. Normal values of PPG are up to 5 mm Hg. Portal pressure expressed as PPG is not affected by changes in the intra-abdominal pressure caused by tense ascites and total volume paracentesis. An increase in intra-abdominal pressure increases both the portal pressure and the IVC pressure but does not significantly affect the PPG (except in the case of marked changes in the intra-abdominal pressure that are associated with changes in splanchnic and systemic hemodynamics).



▪ **Figure 15.10** Isolated fundal gastric varices (IGV-1) in a patient with cirrhosis. (Courtesy of Drs. J. M. Bordas and J. Llach.)

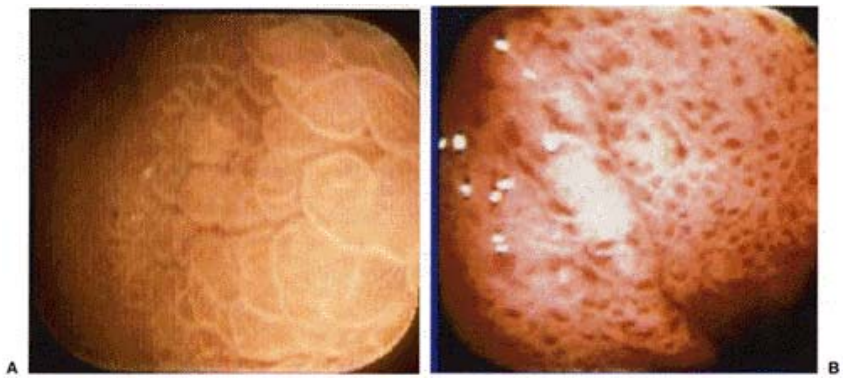
An important concept that has been strongly substantiated in recent years is that the major factor in determining the development of complications and the clinical significance of portal hypertension is an increase in the PPG above a critical threshold value. The threshold value of the PPG for the formation of varices is 10 mm Hg and that for the appearance of other complications, such as variceal bleeding, ascites, and PHG, is 12 mm Hg (1,2,3,4).

Portal pressure can be assessed by direct or indirect methods.

Direct measurement

Direct measurements of portal pressure are invasive investigations based on the surgical, percutaneous transhepatic, or transvenous (transjugular) catheterization of the portal vein. In these techniques, except for the transjugular approach, measurement of the IVC pressure requires the additional and simultaneous puncture of a hepatic vein to determine the PPG. Because of this inconvenience and the associated

surgical or hemorrhagic risk, direct measurements of portal pressure are rarely used. When required, the percutaneous transhepatic approach is the preferred technique (96). Transjugular catheterization of the portal vein is the first step during a transjugular intrahepatic portosystemic shunting (TIPS) procedure. The safety of percutaneous transhepatic or transjugular catheterization of the portal vein can be increased by performing the procedure under ultrasonographic guidance. The hemorrhagic risk is greater in the percutaneous procedure. The risk can be reduced by using a thin needle. This allows one to measure the portal pressure, but not to perform portal venography during the procedure.



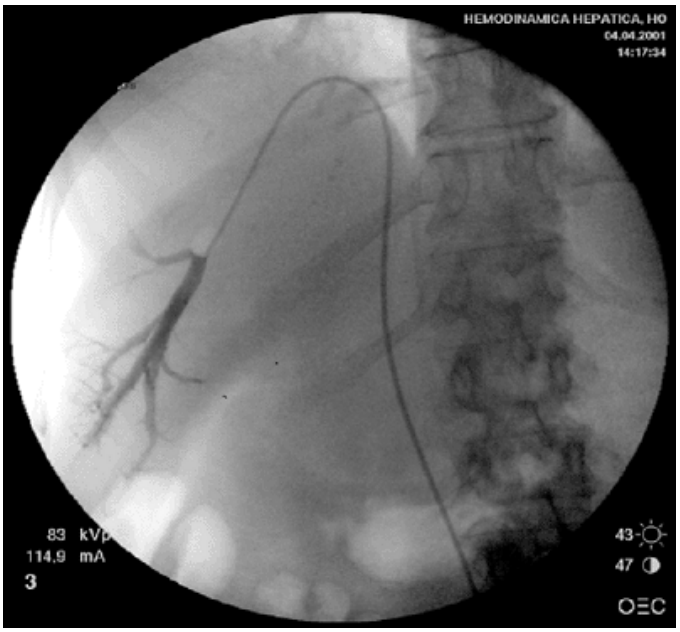
▪ **Figure 15.11** Portal hypertensive gastropathy (PHG). Mild PHG (*left*) is characterized by the mosaic pattern, and severe PHG (*right*) by multiple red spots. (Courtesy of Dr. R. de Franchis and the New Italian Endoscopic Club.)

Indirect measurement

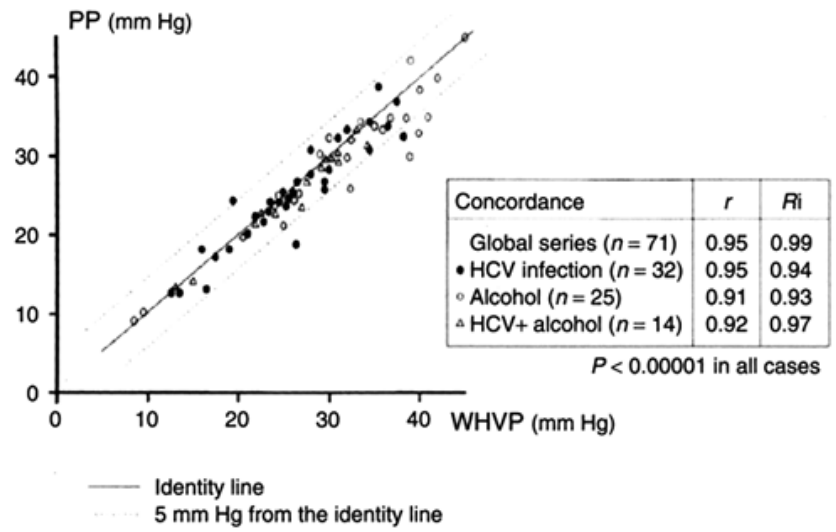
The indirect and safe approach of hepatic vein catheterization, with measurements of the WHVP and FHVP, is the preferred technique to estimate portal pressure (97,98,99).

The FHVP, measured when the tip of the catheter is maintained "free" in the hepatic vein, is close to the IVC pressure (maximal difference of 2 mm Hg). The WHVP is measured by occluding the hepatic vein, either by inflating a balloon at the tip of the catheter (Fig. 15.12) or by advancing the catheter until it becomes "wedged" into a small branch of a hepatic vein. The balloon occlusion technique is preferred because it reflects the pressure of a greater hepatic area than does the "manual occlusion" technique. When blood flow in a hepatic vein is stopped by a "wedged" catheter or a balloon catheter, the static column of blood transmits the pressure to the preceding communicating vascular territory (the hepatic sinusoids). Therefore, the WHVP is a measurement of the hepatic sinusoidal pressure, not of the portal pressure itself. However, it is important to note that the WHVP adequately reflects the portal pressure in diseases causing "sinusoidal" portal hypertension, such as alcoholic liver diseases, nonalcoholic steatohepatitis, and cirrhosis due to hepatitis C virus or hepatitis B virus (Fig. 15.13). These entities are the most frequent forms

of chronic liver disease in developed countries. On hepatic vein catheterization, the PPG is expressed as the HVPG (the gradient between the WHVP and FHVP). The HVPG is the parameter most commonly used to report portal pressure in the medical literature. In the older literature, the HVPG was frequently referred to as *corrected* WHVP or *corrected* sinusoidal pressure.



· **Figure 15.12** Occlusion balloon catheter located in the main right hepatic vein. The wedged hepatic vein pressure (WHVP) is measured after the hepatic vein has been occluded by inflating a balloon at the tip of the catheter. When blood flow is stopped by the balloon, the static column of blood transmits the pressure to the preceding communicating vascular territory (the hepatic sinusoids). Therefore, the WHVP is a measurement of the hepatic sinusoidal pressure, not of the portal pressure itself. The free hepatic vein pressure is measured after the balloon has been released and should be similar to the inferior vena cava pressure.



▪ **Figure 15.13** The wedged hepatic vein pressure (WHVP) accurately reflects the portal pressure (PP) in patients with alcoholic or hepatitis C–related cirrhosis of the liver. (From Perello A, Escorsell A, Bru C, et al. Wedged hepatic venous pressure adequately reflects portal pressure in hepatitis C virus–related cirrhosis. *Hepatology* 1999;30:1393–1397, with permission.)

A limitation of the HVPG is that it does not reflect the PPG in diseases in which the increased resistance is located at presinusoidal sites, such as PVT and some liver diseases predominantly affecting the portal tracts, including schistosomiasis, the initial stages of primary biliary cirrhosis, and idiopathic portal hypertension. In these cases, a direct measurement of the portal pressure may be indicated.

On hepatic vein catheterization, in addition to measuring the HVPG, it is possible to obtain a wedged hepatic retrograde portography using carbon dioxide as a contrast agent. This demonstrates the portal vein in most instances to the point that a failure to demonstrate the portal vein on carbon dioxide retrograde portography strongly suggests the presence of presinusoidal portal hypertension (92).

An additional advantage of this technique is that during hepatic vein catheterization (through a jugular vein), it is possible to obtain a liver biopsy specimen, which adds very little time, discomfort, and risk to the procedure and can be accomplished during a 1-day hospital stay. Furthermore, during hepatic vein catheterization, it is possible to measure the hepatic blood flow with indocyanine green; the intrinsic clearance of indocyanine green is a quantitative test of liver function that assesses overall hepatic metabolic activity (100).

Diseases causing a hepatic outflow block, classified as "postsinusoidal" or "posthepatic" portal hypertension, show abnormal increases in both the WHVP and FHVP, with a normal HVPG.

The main applications of HVPG measurements include evaluation of portal hypertension, assessment of the response to pharmacologic therapy, preoperative evaluation of the risk of resection in patients with small hepatocellular carcinoma (101), prognostic evaluation during variceal bleeding and acute liver failure, and evaluation of the progression of chronic liver disease, especially severe chronic hepatitis C and alcoholic hepatitis, in which the HVPG may be a good index of the response to therapy (102,103).

The main advantages of the hepatic vein catheterization technique are its simplicity, reproducibility, and safety. There was no fatality in more than 10,000 studies. Major complications have been limited to local injury to the femoral or jugular vein (e.g., arteriovenous fistulae, leakage, or rupture of venous introducers). The procedure causes very little discomfort, and if performed under slight conscious sedation, its acceptability is comparable to that of upper gastrointestinal endoscopy. Because of its many clinical applications, hepatic vein catheterization is becoming a routine test in many hospitals. Although it is easy and simple to perform, accurate measurements require specific training because the procedure differs from that used in cardiac catheterization laboratories and intensive care units. It would be desirable if fellowship programs in hepatology included training in this specific technique. The following are a few useful tips to ensure adequate measurements:

1. Use an appropriate scale so that small changes can be detected. The scales used for arterial pressure measurements are not adequate. The scale should be set, at least, so that 1 mm Hg equals 1 mm of paper.

2. Stabilization of venous pressures should be evaluated over a period of at least 1 minute for the WHVP and 15 seconds for the FHVP (some patients may require longer). A slow paper speed should be used in the recorder. A speed of less than 5 mm/second is appropriate.
3. Never rely on digital readings on the screen. These are instantaneous readings and may not be representative of the correct measurement.
4. Calibrate transducers by obtaining tracings of known external pressures at least once a day (e.g., 13.6 cm H_2O should read as 10 mm Hg and 27.2 cm H_2O should read as 20 mm Hg). Discard transducers that do not calibrate exactly.
5. Place the transducer at the level of the right atrium (midaxillary line).
6. Check that the "zero" pressure (with the transducer open to air) corresponds exactly with the "zero" line in the pressure tracing.
7. The FHVP should be measured with the catheter tip inserted less than 5 cm in the hepatic vein. The FHVP should not differ by more than 2 mm Hg from the IVC pressure measured at the level of the hepatic veins.
8. Check that inflating the distal balloon totally occludes the hepatic vein before the WHVP is measured. A slow injection of 5 mL of contrast dye should show the typical "wedged" pattern, without washout through communication with other hepatic veins. Rinse the catheter with a solution of 5% dextrose before the pressures are measured.
9. For each measurement, also obtain a "mean" pressure by "filtering" the waves or by selecting this option in the recorder.
10. Take note of whatever happens. A cough or other movement may cause artifacts that will lead to an incorrect interpretation.

Hepatic Blood Flow

Under normal circumstances, the total hepatic blood flow represents approximately 25% of the cardiac output. One third of this flow is contributed by the hepatic artery and the remainder by the portal vein.

Regulation of hepatic arterial flow

Flow in the hepatic artery is autoregulated. A reduction in portal flow results in an

increase in hepatic arterial flow. Flow in the hepatic artery may increase up to 100% in response to a decrease in portal flow. The mechanism of this "hepatic artery buffer response" is not well known. It has been suggested that it may be mediated in part by adenosine release and washout in the vicinity of the portal tracts (104). The "buffer response" is important when portal blood flow decreases dramatically, as in PVT and portacaval shunting. It has been suggested that after portacaval anastomosis, the prognosis correlates with the increase in arterial flow that follows the diversion of the portal blood flow.

Arterial flow to the liver is not determined by oxygen demand. Under conditions of increased oxygen demand, oxygen extraction rather than arterial flow is augmented in the liver.

Portal vein flow

The portal system is thought to be a passive vascular bed. The portal blood flow is composed of venous blood draining the stomach, intestines, pancreas, spleen, and omentum. Therefore, the factors that govern portal venous flow are predominantly those that control the supply of blood to these organs, such as the ingestion of a meal.

Contrary to the buffer response of the hepatic artery to decreases in portal blood flow, portal venous hyperemia does not occur in response to a decrease in hepatic arterial flow.

Measurement of hepatic blood flow

Clearance techniques

Clearance techniques require the injection of dyes or particles labeled with radioactive isotopes that are avidly extracted by the liver. If a substance is totally extracted by the liver in a single pass, its plasma clearance will be equal to the total hepatic blood flow. However, no substance possesses this property. For this reason, it is necessary to measure the hepatic extraction of the test compound (the fractional uptake in a single passage through the liver) directly. This measurement requires the simultaneous sampling of blood flow in a hepatic vein and a peripheral artery, particularly in the setting of clinical liver disease, in which extraction of the indicator is frequently diminished (100). This represents another application of hepatic vein catheterization. The most commonly used indicator is the albumin-bound dye indocyanine green (105). Bromsulfophthalein and bile salts labeled with radioactive isotopes have also been used. The *extrarenal sorbitol clearance* has been reported to be a safe, noninvasive, and reliable means of measuring "parenchymal" or "effective" hepatic blood flow in patients with a normal or diseased liver (106).

Indicator dilution technique

The indicator dilution technique is an invasive method that requires catheterization of the hepatic artery, portal vein, superior mesenteric artery, or splenic artery (any one of these vessels is sufficient if the splanchnic circulation is normal) and the hepatic vein (107). Its only advantage over clearance techniques is that it does not depend on liver function.

Inert gas washout

The administration of inert gases such as krypton and xenon by intraparenchymal, intrasplenic, or portal vein injection has been used to assess hepatic blood flow. Less invasive methods, such as inhalation and administration through the lumen of the gastrointestinal tract, have also been reported but are not very accurate because it is difficult to assess the exact amount of administered inert gas, which is instantaneously

absorbed. This technique can be adapted to estimate portosystemic shunting.

Measurement of Azygos Blood Flow

This technique is based on the well-known fact that most gastroesophageal collaterals (including esophageal varices) drain into the azygos venous system; therefore, an increased azygos blood flow in patients with portal hypertension reflects the emptying of the collateral blood into the azygos vein (108,109). Obviously, the technique does not allow one to determine the extent of the increase in azygos blood flow that is contributed by esophageal varices and that contributed by periesophageal collaterals, which also drain into the azygos vein. In addition, in 25% of cases, the gastroesophageal collaterals drain into other thoracic veins (i.e., subclavian, innominate, and pulmonary). Therefore, a normal azygos blood flow does not necessarily indicate an absence of esophageal varices. Drainage into other thoracic veins may also be the reason why the azygos blood flow is not significantly correlated with the size of esophageal varices and the risk for bleeding (110).

The direct measurement of azygos blood flow requires retrograde catheterization of the azygos vein with a continuous thermal dilution catheter. This is a simple procedure performed in the course of routine hemodynamic investigations. Fick's principle is applied to measure blood flow by the thermodilution technique. The whole procedure, including repeated measurements of blood flow, takes less than 15 minutes and has been shown to be accurate and reproducible, with less than 10% variability in repeated measurements. Azygos blood flow is usually markedly increased in portal hypertension. Values in healthy subjects range between 0.10 and 0.25 L/minute, whereas in patients with cirrhosis and portal hypertension, these are increased on average to 0.65 L/minute. The azygos blood flow is also increased in patients with noncirrhotic portal hypertension. Azygos blood flow is markedly reduced after surgical portacaval shunting, esophageal tamponade, and orthotopic liver transplantation.

The main application of measurements of azygos blood flow is to monitor the effects of pharmacologic therapy in portal hypertension. The ability of the technique to detect changes in portal–collateral blood flow has increased the understanding of the beneficial effects of the vasoactive drugs currently used in the treatment of portal hypertension. It has been demonstrated that some splanchnic vasoconstrictors, such as propranolol, cause greater decreases in azygos blood flow than in portal pressure. On the other hand, equal falls in portal pressure can be associated with different effects on azygos blood flow. Obviously, a decrease in the HVPG and azygos blood flow, as obtained with propranolol, reflects a greater beneficial effect than a decrease only in the HVPG (as usually happens with vasodilator drugs).

Azygos blood flow can also be measured noninvasively with phase-contrast magnetic resonance angiography (111). Endosonographic pulsed Doppler estimates of azygos blood flow are likely to be much less reliable.

Measurement of Variceal Pressure

The pressure in esophageal varices can be measured using endoscopy by *direct puncture of the varices*, with *endoscopic pressure-sensitive gauges*, or with an *inflating–deflating balloon* attached to the tip of the endoscope (Fig. 15.14). The two latter techniques measure variceal pressure noninvasively, so that the risk for precipitating variceal hemorrhage is eliminated. Variceal puncture is justified only by endoscopic injection sclerotherapy.

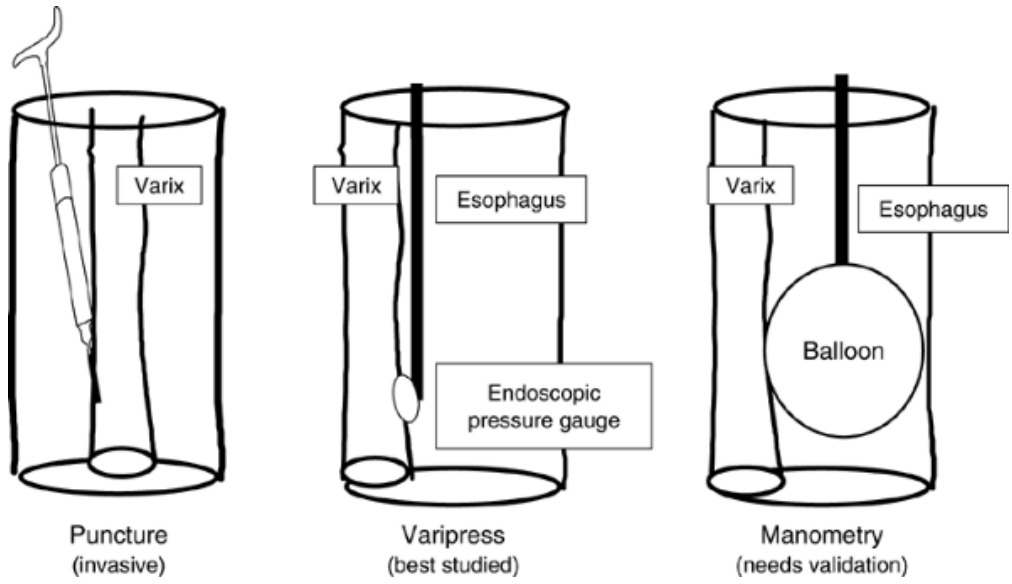
Noninvasive techniques are based on the assumption that because of their thin walls and lack of external tissue support, varices behave as elastic structures; therefore, the pressure needed to compress a varix (which can be sensed by a pressure gauge or directly visualized using a clear balloon) equals the pressure inside the varix. Several studies have shown a good correlation between these techniques and the more

aggressive variceal puncture technique (2,112).

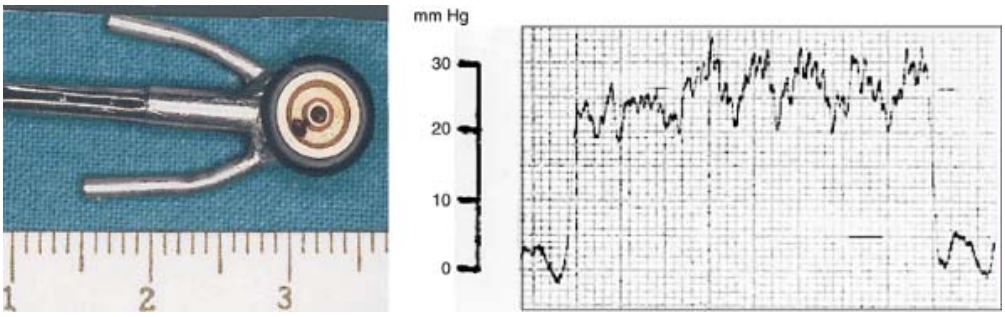
The endoscopic pressure gauge is the method used most commonly in prospective studies. A pressure-sensitive capsule, attached to the tip of an endoscope, consists of a small chamber covered by a thin latex membrane that is continuously perfused with nitrogen. The perfusion pressure is continuously recorded by means of an electromagnetic pressure transducer. It is assumed that when the gauge is applied over a varix, the increase in pressure required to perfuse the gauge equals the pressure inside the varix. The difference between the pressure measured when the gauge is applied over a varix and when the gauge is free in the esophageal lumen equals the transmural variceal pressure, which is the value used to express results (113).

Endoscopic pressure measurements can be difficult, especially in small varices. However, these techniques are reproducible when strict criteria are used to define satisfactory measurements, such as the following:

(a) Stable intraesophageal pressure, (b) absence of artifacts caused by esophageal peristalsis, and (c) correct placement of the capsule over the varix, demonstrated by fine fluctuations of the pressure tracing according to the cardiac cycle or respiration, for at least 10 seconds or three respiratory cycles, respectively (114,115) (Fig. 15.15). These conditions are easily met when the procedure is performed by a skilled endoscopist in a cooperative patient. The administration of 20 mg of butyl scopolamine intravenously diminishes the artifacts caused by esophageal peristalsis without affecting variceal pressure measurements (116). Nonetheless, approximately 25% of patients initially scheduled for variceal pressure measurements must be excluded because of technical difficulties that preclude obtaining correct measurements. Of these, two thirds are patients with small varices.



▪ **Figure 15.14** The pressure of esophageal varices can be measured at endoscopy by direct puncture (*left*), with an endoscopic pressure gauge (*center*), or with an inflating–deflating balloon attached to the tip of the endoscope (*right*).



▪ **Figure 15.15** Endoscopic pressure gauge (Varipress, Labotron, Barcelona, Spain) and a typical recording of variceal pressure showing the fine fluctuations of the tracing according to the cardiac cycle or respiration.

Studies in which variceal pressure was measured in patients with cirrhosis have provided important information. It has been shown that variceal pressure is significantly lower than portal pressure (2,112). This difference is thought to be a consequence of resistance to portal–collateral blood flow in the collaterals feeding the varices and indicates that measurements of variceal pressure cannot be used to estimate portal pressure. It has also been shown that variceal pressure is greater in patients whose varices have bled than in those whose varices have not bled, a finding that supports the concept that increased variceal pressure plays a key role in the mechanism of bleeding and suggests that measurements of variceal pressure may be of prognostic value in assessing the risk for bleeding. Repeated measurements of variceal pressure can detect acute and long-term changes in variceal pressure produced by the administration of pharmacologic agents.

It has been demonstrated that drugs that decrease portal pressure cause equal or even greater falls in variceal pressure. A reduction of variceal pressure, after pharmacologic therapy, of at least 20% from baseline has been associated with a low actuarial probability of variceal bleeding on follow-up (7% at 3 years vs. 46% in patients without this reduction) (117).

Endosonography

Endosonography (or endoscopic ultrasonography) allows the visualization of esophageal and gastric varices, periesophageal and perigastric collateral veins, the portal venous system, and the azygos vein. Endosonography, however, does not seem to add further important prognostic information about the risk for the complications of portal hypertension in comparison with conventional endoscopy (which also identifies red color signs in the variceal wall) (118,119).

The clinical use of endosonography is restricted to two main applications: The diagnosis of gastric fundal varices when the results of endoscopy are doubtful and assessment of the risk for recurrence after varices have been eradicated by endoscopic sclerotherapy or banding ligation. In the latter situation, the finding of grossly dilated periesophageal veins or patent perforating veins below the gastroesophageal junction carries a high risk for the recurrence of varices.

The combined use of endosonography for an objective measurement of variceal diameter and endoscopy for the measurement of transmural variceal pressure allows a

quantitative estimation of variceal wall tension, which is the relevant parameter in regard to risk for variceal bleeding. This technique has proved useful in pathophysiologic and pharmacologic research in patients with portal hypertension.

Clinical Consequences of Portal Hypertension

The portal hypertensive syndrome portal hypertensive gastropathy is responsible for many of the manifestations of advanced, decompensated liver disease. Some of these complications are a direct consequence of portal hypertension: Gastrointestinal bleeding resulting from ruptured gastroesophageal varices, portal hypertensive gastropathy, and colopathy; hyperkinetic syndrome; hypersplenism; and an increased systemic availability of drugs and endogenous compounds with rapid hepatic uptake (5). In other complications, portal hypertension plays a key role, although it is not the only pathophysiologic factor involved in their development. These include ascites, abnormalities of renal function, hepatorenal syndrome, spontaneous bacterial peritonitis, hepatopulmonary syndrome, and hepatic encephalopathy. This chapter focuses on gastrointestinal bleeding; the other complications are dealt with elsewhere in this book.

Collateral Circulation

The most important clinical consequences of portal hypertension are related to the formation of portosystemic collaterals; these include gastroesophageal varices, which are responsible for the main complication of portal hypertension, massive upper gastrointestinal bleeding (120).

Collaterals develop in areas where anatomic connections exist between the portal venous and systemic circulation. The portal system and the systemic venous circulation are connected at several locations (120). Gastroesophageal collaterals develop from connections between the short gastric and coronary veins and the esophageal, azygos, and intercostal veins; the result is the formation of esophageal and gastric varices. Other collaterals may also develop: Between the superior hemorrhoidal vein and the middle and inferior hemorrhoidal veins, giving rise to anorectal varices; between the portal and epigastric veins through the reopening of remnants of the umbilical or paraumbilical veins, forming a vascular net that is at times apparent on the abdominal wall as a caput medusae and causing a murmur over the umbilicus (the Cruveilhier-Baumgarten syndrome); between the portal system and the posterior abdominal wall through the liver capsule and diaphragm; and between the portal system and the left renal vein, forming spontaneous splenorenal shunts. In instances of PVT, "hepatopetal" collaterals develop between the splenic and the coronary veins through the short gastric veins, giving rise to gastric varices, and between the mesenteric or portal vein and the intrahepatic vena porta through the veins of Sappey, causing pseudocavernomas of the portal vein (120). Ectopic varices may develop at other locations, depending on local anatomic factors. Most ectopic varices develop in the duodenum (primarily associated with extrahepatic portal hypertension) and in the colon and small intestine (more frequent in patients who have previously undergone abdominal surgery). Overall, these ectopic varices account for between 1% and 5% of all episodes of variceal bleeding (121,122).

Lymph flow is also increased in portal hypertension. Enlargement of the hilar lymphatics and lymph nodes has been demonstrated. Increased hepatic lymph flow contributes to the formation of ascites (123).

Natural History and Clinical Manifestations of Portal Hypertension

The information provided here on the natural history and clinical manifestations of portal hypertension is

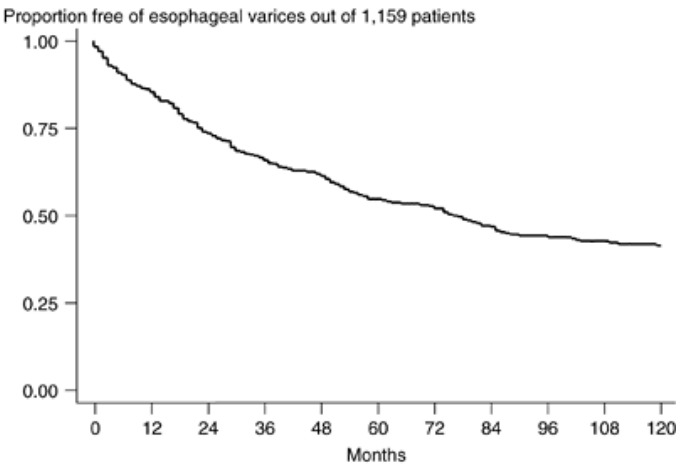
drawn from liver cirrhosis, the best studied disease causing portal hypertension. It is generally accepted that this information is applicable to most of the other causes of portal hypertension, although some differences may be identified in specific disease. These are outlined wherever needed.

Development of esophageal varices

Portal hypertension is an almost unavoidable complication of cirrhosis. Prevalence of varices at the time of the diagnosis of cirrhosis is widely variable across published studies and ranges from 0% to 80% in 93 prognostic studies published from 1980 to 2003. Overall, the median prevalence of esophageal varices in these studies is 55%; in studies including mostly compensated patients it is 48% and in those including mostly decompensated patients it is 61% (124,125).

Recent prospective cohort studies showed that the incidence of esophageal varices in patients with newly diagnosed cirrhosis is nearly 5% per year (85,126,127). A similar finding was recently also reported in a large consensus conference after a thorough review of available studies (128). Therefore, assuming that approximately 40% of patients already have varices when first seen, the estimated risk of varices is of approximately 65% at 5 years after the diagnosis of cirrhosis (Fig. 15.16).

Cross-sectional studies have shown that esophageal varices do not develop below the threshold HVPG value of 10 mm Hg (4). However, not all patients with an HVPG above this threshold have esophageal varices and the relationship between the incidence of varices and HVPG above this level is poorly understood. Worsening of liver function and continuing exposure to alcohol are clinical factors associated with an increasing risk of developing varices. The HVPG frequently increases with worsening liver function and continued alcohol abuse and may decrease when liver function improves and alcohol is discontinued (129). A baseline HVPG greater than 10 mm Hg (clinically significant portal hypertension) is independently associated with an increased risk of developing varices (85,128). Because of this, an increased HVPG is presently considered the most important risk factor for the development of esophageal varices.



▪ **Figure 15.16** The incidence of esophageal varices among 1,159 patients with cirrhosis and without varices at diagnosis. Cumulative data from two studies on the natural history of cirrhosis.

Progression of esophageal varices from small to large

Once developed, varices increase in size from small to large before they eventually

rupture and bleed. Prospective studies have reported progression rates ranging from 0.05 to 0.20 per year (median 0.12) (126,127,130). This variability probably reflects different patient selection across studies with inclusion of patients with different degrees of progression of the underlying cirrhosis. In this respect it may be worth noting that the only study that included only those patients with newly diagnosed cirrhosis reported a variceal progression rate of 0.05 per year.

Improvement in liver function and abstinence from alcohol may result in decrease or even disappearance of varices (129). As already stated, this may be related to a decrease in HVPG. It has been shown that changes in HVPG (either "spontaneous" or caused by drug therapy or TIPS) are usually accompanied by parallel variations in the size of the esophageal varices, which is significantly reduced when HVPG decreases below 12 mm Hg (131) or when it decreases more

than 15% from baseline value. Therefore, the increased HVPG plays a key role both in the development and progression of the varices. It will probably become a main target in monitoring treatment to prevent variceal bleeding or rebleeding.

A striking association has been noted between an increased size of esophageal varices and a poor prognosis of cirrhosis in terms of risk of bleeding, of developing ascites, and mortality (Fig. 15.17).

Incidence of first bleeding from esophageal varices and indicators of risk

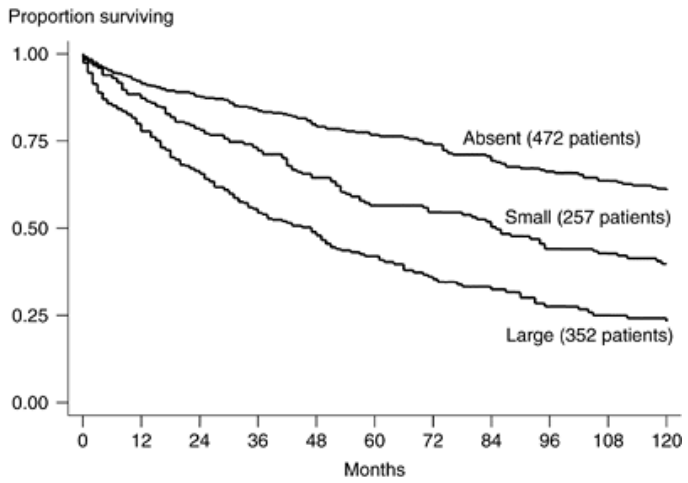
The probability of bleeding from esophageal varices is variable and depends on cirrhosis status. In patients with no varices at endoscopy, the risk of bleeding is as low as 1% to 2% per year (85,125,127) and increases to approximately 5% to 6% per year in those with small varices (Fig. 15.18) and to 15% per year in those with medium or large varices at diagnosis (125) (Fig. 15.18).

The importance of the size of the varices in determining the bleeding risk is further discussed in the subsequent text.

Clinical and endoscopic risk indicators:

Among patients with esophageal varices the bleeding incidence is significantly lower in Child-Pugh class A patients than in class B or C patients, and in patients without ascites versus those with ascites, independent of variceal size (125).

The risk of variceal bleeding is significantly associated with variceal size, severity of liver dysfunction expressed by the Child-Pugh classification, and red weal marks. These risk indicators have been combined in the North Italian Endoscopic Club (NIEC) index (90,91), which allows the classification of patients into different classes, with predicted 1-year bleeding risk ranging from 6% to 76%. Although this index has been validated (91), its predictive accuracy is far from satisfactory. In fact, the best operative characteristics of the NIEC index in the prediction of the bleeding risk are 74% sensitivity and 64% specificity. Another endoscopic index combining variceal size, the presence of PHG, and the presence of gastric varices has similar unsatisfactory operative characteristics for the prediction of bleeding risk. Whether these indexes can be improved by incorporating additional parameters related to portal hypertension (i.e., spleen size, platelet count) remains to be determined.



▪ **Figure 15.17** Survival of patients with cirrhosis according to the presence and size of esophageal varices at diagnosis. Cumulative data from two studies of the natural history of cirrhosis. *Absent*, no esophageal varices at diagnosis; *small*, small esophageal varices at diagnosis; *large*, medium or large varices at diagnosis.

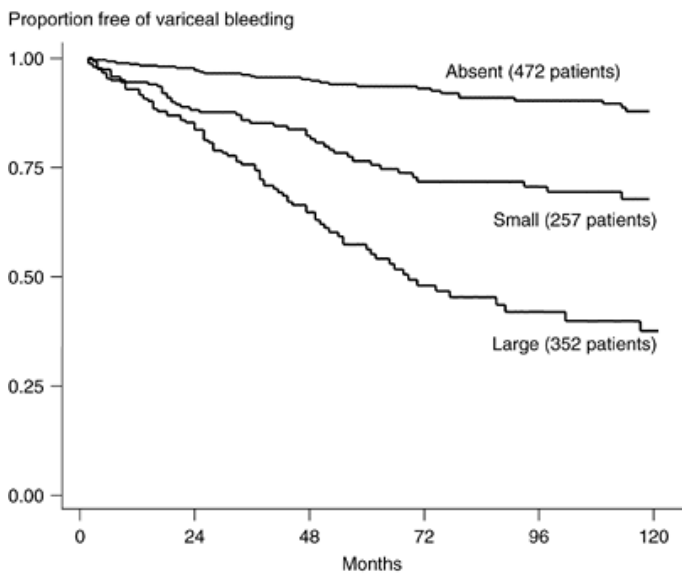
Hemodynamic risk indicators:

Cross-sectional and prospective studies have shown that esophageal varices do not bleed below the threshold HVPG level of 12 mm Hg (128). Importantly, it has also been demonstrated that variceal hemorrhage does not occur if HVPG is reduced, either spontaneously or pharmacologically, to levels below 12 mm Hg (128) or more than 20% from baseline. The reduction in risk of bleeding occurs in spite of varices continuing to be demonstrated in most patients, but variceal size is significantly decreased when HVPG is reduced below threshold values (1,85).

Variceal pressure is significantly related to the risk of bleeding and death (113).

The relationship between variceal pressure and the risk for bleeding reflects the increase in variceal wall tension associated with increasing

variceal pressure and size (2,132). Variceal wall tension cannot be measured accurately because the endoscopic evaluation of variceal diameter is only approximate, and it is not possible to measure variceal wall thickness with sufficient accuracy. However, it has been suggested that endosonography or high-resolution endoluminal probe sonography allow a more precise and reliable measure of variceal diameter than endoscopy (133,134) and may allow the estimation of wall thickness. This may contribute to further improvement in the assessment of the risk of variceal bleeding (117).



▪ **Figure 15.18** Incidence of variceal bleeding in patients with cirrhosis according to the presence and size of esophageal varices at diagnosis. Cumulative data from two studies of the natural history of cirrhosis. *Absent*, no esophageal varices at diagnosis; *small*, small esophageal varices at diagnosis; *large*, medium or large varices at diagnosis.

Ultrasonographic risk indicators:

Although great efforts have been made, no ultrasonographic or Doppler flowmetry parameters have been identified as reliable indicators of the risk of variceal bleeding (128).

Assessment of the risk of first bleeding from esophageal varices in clinical practice

The size of varices, as estimated at endoscopy, is the most widely used risk indicator of first variceal bleeding. Patients with medium- to large-sized varices are considered to be at considerable risk of bleeding and should receive therapy to prevent variceal bleeding (128,135). Patients with small varices but with advanced liver failure (Child-Pugh C) have a similar bleeding risk (90,91).

Screening for esophageal varices:

In a recent large consensus conference it was agreed that all patients with cirrhosis should be screened for the presence of esophageal varices at the time of initial diagnosis (8).

Although several studies indicate that noninvasive tests (particularly, platelet count and data obtained from abdominal ultrasonography) may have a potential use in selecting patients at high risk for varices, so far none of these have been proved to be accurate enough that endoscopy can be safely omitted in patients with negative noninvasive indicators (132).

Timing for subsequent evaluations:

In patients without varices on initial endoscopy, a second (follow-up) evaluation should be performed to detect the development of varices before they bleed.

Because the expected incidence of newly developed varices is approximately 5% per year, the general consensus (125,126,127,128) is that endoscopy should be repeated after 2 to 3 years in patients without varices on the first endoscopy. In patients with small varices on initial endoscopy the aim of subsequent evaluations is to detect the progression of small to large varices because of its important prognostic implications. On the basis of an expected 10% to 15% per year rate of progression of variceal size, endoscopy should be repeated every 1 to 2 years in patients with small varices (125,126,127,128).

Acute bleeding from esophageal varices: Definitions and intervals

Ruptured esophageal varices cause 60% to 70% of all upper gastrointestinal bleeding episodes in patients with portal hypertension (136). Variceal bleeding is diagnosed on emergency endoscopy. Diagnosis is based on observing: (a) Blood spurting from a varix (near 20% of patients), (b) white nipple or clot adherent on a varix, and (c) varices without other potential sources of bleeding. Because variceal bleeding is frequently intermittent, it is difficult to assess when the bleeding stops and when a new hematemesis or melena should be considered a rebleeding episode. Several consensus conferences have addressed this issue and set definitions for events and timing of events related to the variceal bleeding episode (8,137). The time frame for the events related to the acute bleeding episode is 5 days (8). The index bleeding episode is separated from the first episode of rebleeding by at least a 24-hour bleeding-free period, during which there is no new hematemesis and/or melena with stable hemoglobin level (137). Using these criteria the median duration of the acute variceal bleeding episode is approximately 18 hours (136). Data from placebo-controlled clinical trials have shown that variceal bleeding ceases spontaneously in 40% to 50% of patients. Active treatment achieves control of bleeding within 24 hours of admission in nearly 80% of episodes (138). Active bleeding on endoscopy (138), bacterial infection (139), HVPG greater than 20 mm Hg, hematocrit, aspartate aminotransferase, Child-Pugh class, and PVT (128,136) are significant prognostic indicators of failure to control bleeding. Immediate mortality from uncontrolled bleeding is in the order of 5% (136).

Early rebleeding is significantly associated with the risk of death within 6 weeks, suggesting that its prevention should be a primary objective in the therapeutic approach to variceal bleeding. The incidence of early rebleeding ranges between 30% and 40% in the first 6 weeks. The risk peaks in the first 5 days, with 40% of all rebleeding episodes occurring in this very early period, remains high during the first 2 weeks and then declines slowly in the next 4 weeks. After 6 weeks the risk of further bleeding becomes virtually equal to that before bleeding (140).

Active bleeding in emergency endoscopy, gastric varices, low albumin and/or high blood urea nitrogen (BUN) levels, and HVPG greater than 20 mm Hg have been reported as significant indicators of early rebleeding risk (141).

Six-week mortality— because it may be difficult to assess the true cause of death (i.e., bleeding vs. liver failure or other adverse events), the general consensus is that any death occurring within 6 weeks from hospital admission for variceal bleeding should be considered a bleeding-related death. Six-week mortality after variceal bleeding is approximately 20%. Almost 40% of deaths are caused by uncontrolled bleeding, either during the initial episode or after early rebleeding (136). Like rebleeding, mortality peaks in the first few days after bleeding, slowly declines thereafter, and after 6 weeks becomes constant and similar to that before bleeding (136,140).

Accurate indicators of early death risk could allow selection of patients for emergency shunt or TIPS before their conditions deteriorate, hampering further therapy.

Unfortunately, the risk indicators so far identified are mainly indicators of poor liver and/or renal function, which are also associated with high operative risk and are consequently of limited clinical value. On hospital admission, the most consistently reported death risk indicators are Child-Pugh classification or its components, BUN or creatininemia, age, active alcohol abuse, active bleeding on endoscopy, bacterial infection, HVPG greater than 20 mm Hg, hepatocellular carcinoma, and the total number of blood units transfused (136).

Long-term recurrent bleeding from esophageal varices and mortality

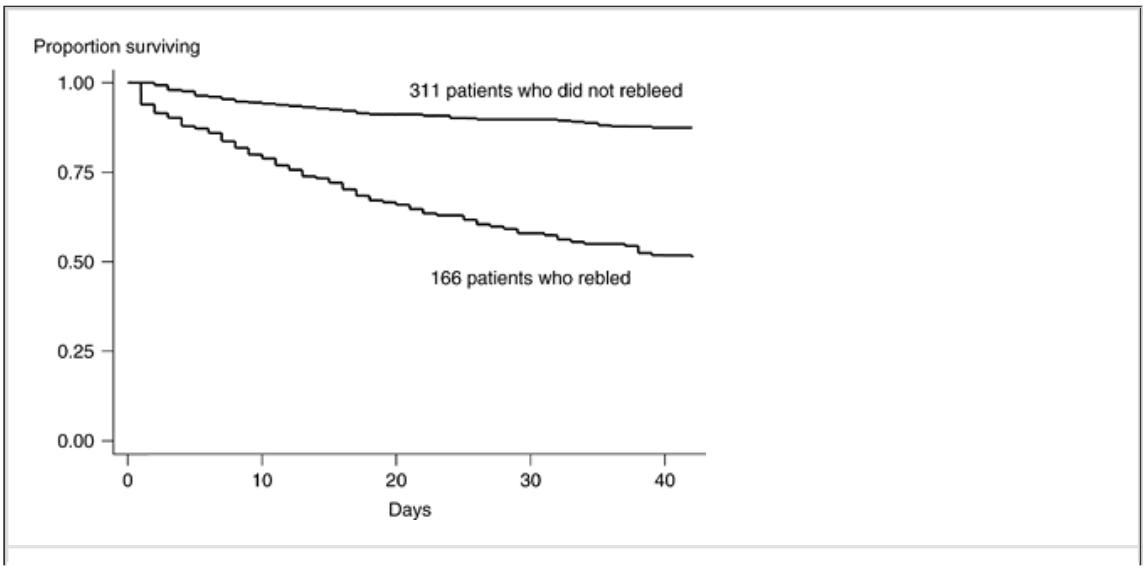
Patients surviving a first episode of variceal bleeding have a very high risk of rebleeding and death. In untreated controls in randomized clinical trials (RCTs), reported after 1981, of nonsurgical treatment for prevention of recurrent bleeding, the median rebleeding incidence within 1 to 2 years of the study, was 63% (141). The corresponding mortality figure was 33% (Fig. 15.19). Because of these high risks, all patients surviving a variceal bleeding should be treated for prevention of rebleeding independent of other risk indicators (142).

RCTs for prevention of rebleeding suggest that the risk indicators of rebleeding and death are variceal size, Child-Pugh class, continued alcohol abuse and hepatocellular carcinoma. HVPG greater than 20 mm Hg is significantly associated with a higher risk of 1-year mortality. Reduction of HVPG to below 12 mm Hg totally prevents recurrent bleeding (143). A reduction in HVPG by more than 20% of baseline values is associated with reduced risk of rebleeding, ascites, spontaneous bacterial peritonitis, and death (144).

Gastric varices

The natural history of gastric varices is far less known than that of esophageal varices. According to the most widely used classification (145,146) gastric varices may be found as a continuation of esophageal varices along the lesser curve of the stomach (gastroesophageal

varices type 1 [GOV1]) or in the fundus (GOV2); more rarely *isolated gastric varices* (i.e., not connected with esophageal varices) may be found in the fundus (isolated gastric varices type 1 [IGV1]) or in the rest of the stomach (IGV2). These are more frequent in patients with prehepatic portal hypertension. Overall, the prevalence of gastric varices in patients with portal hypertension is approximately 20% (14% GOV1, 4% GOV2, 2% IGV1 or 2) (146,147). Sometimes it may be difficult to differentiate gastric varices from plicae; endoscopic ultrasonography may be helpful in these cases.



▪ **Figure 15.19** Survival after an episode of variceal bleeding according to early rebleeding. Cumulative proportion of survivors among a cohort of 477 patients with esophageal variceal bleeding observed consecutively at the Medical Department of V. Cervello Hospital, Palermo, Italy, between 1984 and 1992. The survival of patients who experienced early rebleeding was significantly worse.

Gastric varices are commonly fed through the short and/or posterior gastric veins and are frequently associated with lower portal pressure than are esophageal varices. Moreover, spontaneous shunts may develop between the splenic vein (i.e., splenorenal shunt or gastric varices) and the left renal vein through the inferior phrenic or suprarenal vein, respectively. Such shunts are termed *gastrorenal shunts* and may be found in 60% to 85% of cases (146). For these reasons, the risk of bleeding of gastric varices is generally lower than that of esophageal varices, but it is associated with a higher incidence of portosystemic encephalopathy.

Gastric varices are the source of 5% to 10% of all upper digestive bleeding episodes in patients with cirrhosis (136). The 2-year risk of bleeding has been reported to be markedly lower from gastric nonfundal varices than from fundal varices: 10% for GOV1 or IGV2, 78% for IGV1, and 55% for GOV2 (136,145). As for esophageal varices, the risk of bleeding is significantly related with the variceal size, Child class, and the presence of red color signs on the varices.

The effect of sclerotherapy or banding ligation of esophageal varices on gastric varices is still unsettled: Regression, no change, and de novo appearance of gastric varices have all been reported.

Bleeding-related mortality after a first gastric variceal bleeding episode is approximately 20%, and long-term recurrent bleeding and mortality are in the same order as those for esophageal varices.

Portal hypertensive gastropathy

Gastric mucosal changes associated with portal hypertension (148,149,150) are termed *PHG*. The most frequently observed elementary lesions of PHG are the "mosaic pattern" and the "cherry red spots." The mosaic pattern consists of multiple erythematous areas outlined by a white reticular network and is generally considered as "mild" PHG. Cherry red spots are round, red lesions, slightly raised over the surrounding hyperemic mucosa. These carry a higher bleeding risk and are considered to reflect "severe" PHG (118). Reliability and clinical relevance of this classification as mild or severe PHG (147) have been recently validated (151). The term *PHG* has been used after its relationship with portal hypertension was definitively recognized. The gastric mucosal changes of PHG are associated with increased gastric mucosal and submucosal perfusion and, therefore, are hyperemic—not "congestive"—changes. The term *congestive gastropathy* is therefore inadequate and should not be used (149,152). Histologically, these lesions are characterized by dilatation of the capillaries and venules of the gastric mucosa, whereas mucosal inflammation and *Helicobacter pylori* infection are infrequent (153).

Data on prevalence, incidence, and natural history of PHG differ widely across studies (154), mostly because patients were included at different stages of progression of cirrhosis.

During the diagnosis of cirrhosis, the prevalence of PHG is approximately 30% and its incidence is approximately 12% per year (155). However, in patients with advanced cirrhosis these figures may be as high as 70% and 30%, respectively (147). Patients

with severe liver dysfunction and large esophageal varices are at higher risk of developing PHG, whereas large fundal varices may have a protective role, particularly when they are associated with spontaneous gastrorenal shunt. Endoscopic variceal sclerotherapy or banding has been reported as a risk factor for PHG (154). Overall, during the course of cirrhosis, mild PHG may be observed in up to 50% to 70% of patients and severe PHG in 20% to 40% (155). Progression of severity, and also disappearance of PHG, have both been reported (147,156).

The clinical course of PHG is characterized by overt or chronic gastric mucosal bleeding. The incidence of overt bleeding from any source in patients with mild PHG is approximately 5% per year, as compared to 15% for severe PHG. The source of bleeding is the gastric mucosa in most of these bleeding episodes. Overt bleeding from PHG is usually manifested by melena and has a far better prognosis than variceal bleeding, with less than 5% mortality per episode. Mortality is higher in patients with severe PHG, but this has been found to be dependent on the severity of liver dysfunction (155).

The incidence of minor mucosal blood loss, without overt bleeding, is approximately 8% per year in patients with mild PHG and up to 25% in those with severe PHG, in whom severe chronic iron deficiency anemia may result, requiring frequent hospital admissions and blood transfusions. It appears that the wide use of β-blockers in patients with cirrhosis is reducing both chronic and overt bleeding from PHG because it has been proved that β-blockers significantly reduce the rebleeding risk in patients who have bleeding from PHG (157,158).

PHG should be distinguished from gastric antral vascular ectasia (GAVE). This is a distinct entity that may be found in association with conditions different from cirrhosis, such as scleroderma or chronic gastritis. GAVE is characterized by aggregates of red spots, usually with radial distribution from the pylorus in the antrum of the stomach (for this pattern it is also called *watermelon stomach*). Histology of GAVE is characterized by marked dilatation of capillaries and collecting venules in the gastric mucosa and submucosa, with areas of intimal thickening in fibromuscular hyperplasia, fibrohyalinosis, and thrombi (154). From a clinical point of view, GAVE behaves as severe PHG, but it may be less responsive to β-blocker treatment.

Portal hypertensive lesions in the gut

Mucosal lesions similar to those of PHG have been described in the duodenum, jejunum, and colon. Similar to PHG, these lesions tend to cause overt or chronic occult mucosal bleeding. Their incidence and clinical course have not been adequately studied. These lesions are managed similarly as PHG.

Pathophysiologic Basis of Therapy

Clinical–Hemodynamic Correlations in Portal Hypertension

Several studies have demonstrated that in patients with cirrhosis who receive either placebo or long-term pharmacologic therapy for portal hypertension, the occurrence of clinical events is related to the hemodynamic changes observed during treatment. Therefore, changes in the HVPG before and during maintenance therapy (or no treatment) are closely correlated with the occurrence of first variceal bleeding or rebleeding. These studies have demonstrated that the HVPG response to treatment can be considered "good" when the HVPG decreases by at least 20% of the baseline value. This response is associated with a very low risk for rebleeding (<10% at 2 years) (Fig. 15.20). The HVPG response is "optimal" when the HVPG decreases to 12 mm Hg or below, in which case the risk for variceal bleeding is negligible (131,143). An optimal response is also associated with a significant reduction in the size of varices and a significantly longer actuarial survival (131). Therefore, these studies show that

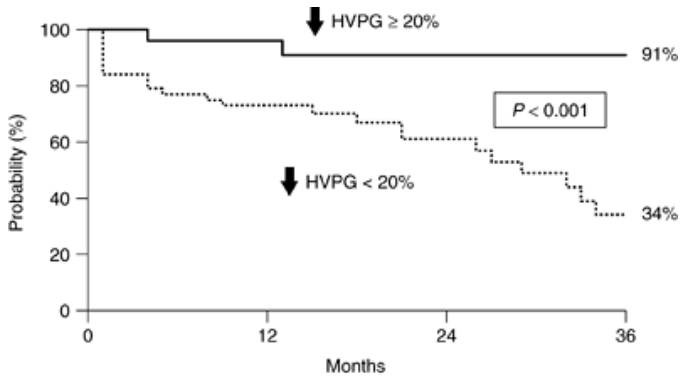
decreasing the portal pressure below the critical threshold values for clinically significant portal hypertension is associated with a reversal of the natural history of the portal hypertensive syndrome and an improved prognosis. These findings represent the rational basis for the medical treatment of portal hypertension.

Therefore, the goal of long-term pharmacologic therapy in patients with portal hypertension should be to decrease the HVPG by at least 20% from baseline values, and preferably to below the threshold of 12 mm Hg. The rational treatment of portal hypertension requires that the underlying alteration—the increase in HVPG—be modified. Such modification should be based on an understanding of the mechanisms that lead to increased portal pressure in liver disease.

Pharmacologic Manipulation of the Intrahepatic Circulation

Studies have shown that a relative deficit of NO and an exaggerated response to endogenous vasoconstrictors

(e.g., norepinephrine, ET, angiotensin II) are responsible for approximately 30% to 40% of the increased intrahepatic vascular resistance of cirrhosis; therefore, some pharmacologic approaches aim at decreasing intrahepatic resistance (Fig. 15.21).



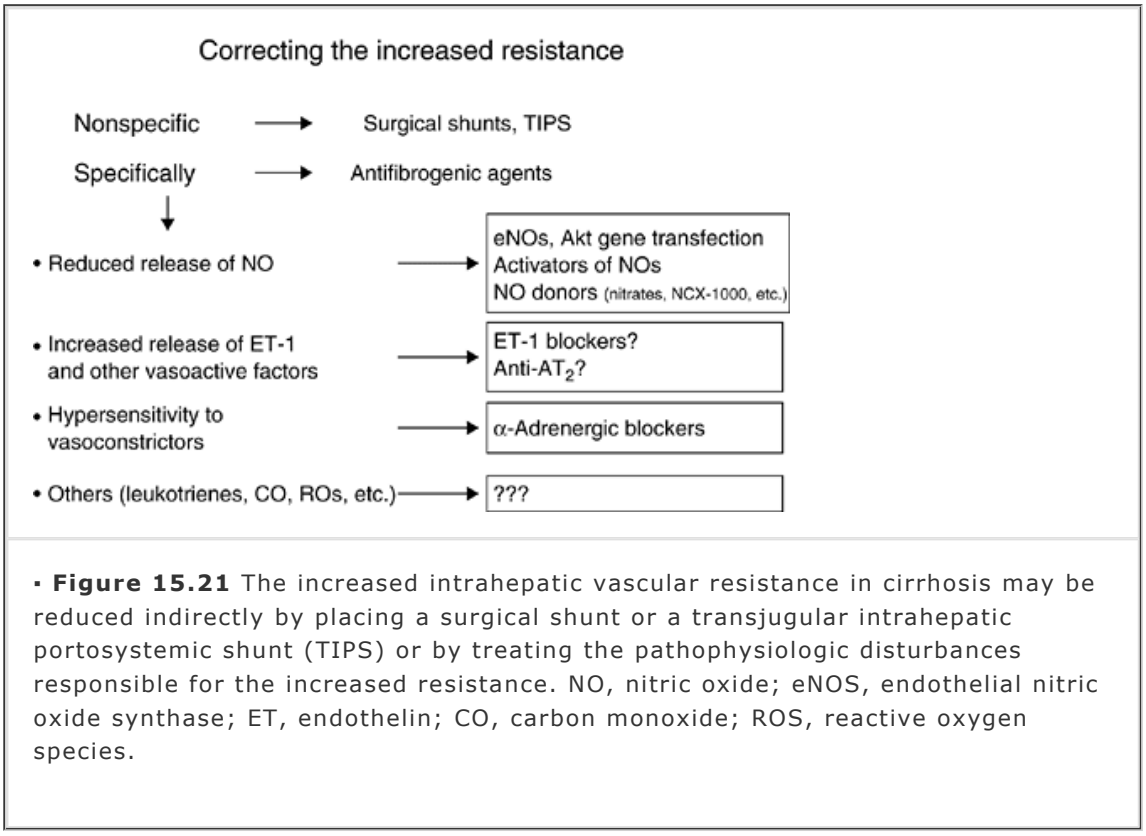
▪ **Figure 15.20** Actuarial probability of remaining free of variceal rebleeding according to the hemodynamic response of the hepatic vein pressure gradient (HVPG) to pharmacologic treatment. A reduction in the HVPG of at least 20% of the baseline values is associated with a very low risk for rebleeding (<10% at 3 years). When the portal pressure gradient decreases to 12 mm Hg or below, the risk for variceal bleeding is negligible. (From Feu F, Garcia-Pagan JC, Bosch J, et al. Relation between portal pressure response to pharmacotherapy and risk of recurrent variceal hemorrhage in patients with cirrhosis. *Lancet* 1995;346:1056–1059, with permission.)

Increasing intrahepatic delivery of nitric oxide

Nitrovasodilators such as NTG, isosorbide dinitrate (ISDN), and IMN, through their capacity to release NO, may compensate for the NO deficit within the cirrhotic liver and thereby reduce the intrahepatic vascular resistance. IMN, the most extensively studied nitrate, has been shown to reduce HVPG in patients with cirrhosis without reducing the hepatic blood flow; its effect reflects a reduction in the hepatic vascular resistance (159). Although it is possible to determine the effect of pharmacologic agents on the individual factors that determine portal pressure (i.e., resistance and flow) in the

experimental setting, it is not easy to do so in clinical studies. A further complication is the fact that the vasodilator action of these drugs is not limited to the hepatic and portal circulation but extends to the systemic circulation, where they cause arterial hypotension; this in turn may elicit reflex splanchnic vasoconstriction, with an ensuing reduction in portal blood flow (160). Vasodilators usually also decrease the cardiac preload and hence the cardiac output, which may further decrease the splanchnic

blood flow. Moreover, many vasodilators reduce the vascular resistance in the portal–collateral circulation. This represents another mechanism by which a vasodilator may reduce the PPG, but at the expense of increased blood flow in the portosystemic collaterals and esophageal varices (134). In some instances, the beneficial effect of decreasing intrahepatic resistance by means of a vasodilator, in terms of a reduction in portal pressure, is offset by the splanchnic vasodilator effect of the drug, which increase the portal–collateral blood flow and prevent any decrease in portal pressure (161).



▪ **Figure 15.21** The increased intrahepatic vascular resistance in cirrhosis may be reduced indirectly by placing a surgical shunt or a transjugular intrahepatic portosystemic shunt (TIPS) or by treating the pathophysiologic disturbances responsible for the increased resistance. NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ET, endothelin; CO, carbon monoxide; ROS, reactive oxygen species.

Another important limitation of vasodilators is that they may be dangerous in patients with advanced cirrhosis. These drugs, by enhancing preexisting peripheral vasodilatation, further decrease the arterial blood pressure and activate endogenous vasoactive systems, which may worsen sodium retention and ascites. However, recent studies have shown that in patients with compensated cirrhosis, long-term treatment with IMN is safe when combined with β-blockade (162). Combined treatment, unlike treatment with IMN alone, does not cause adverse effects on renal function, sodium handling, or endogenous vasoactive systems because the mild systemic vasoconstriction and suppression of renin release resulting from β-blockade oppose the adverse systemic and renal effects of IMN.

Another method to increase the intrahepatic production of NO is to administer agents that act preferentially in the liver, such as the "liver-selective" NO donor NCX-1000 (163,164,165,166). Interest in this method has increased because of recent studies showing that in experimental models of cirrhosis the expression of NO synthase in liver cells can be enhanced by the portal injection of adenovirus transfected with the gene

encoding neural NOS or eNOS and that such treatment may significantly reduce portal pressure for some weeks (52,164,165). Similarly, transfer of the constitutively active Akt results in enhanced eNOS activity and reduces portal pressure in carbon tetrachloride–induced cirrhotic rats (53). More recently, it has been shown that hepatic eNOS activity may be enhanced in patients with cirrhosis by the administration of simvastatin (54,166), findings that may be of therapeutic relevance.

α-Adrenergic antagonists

Prazosin is an α_1-adrenergic antagonist that markedly reduces the HVPG in patients with cirrhosis. This reduction is associated with an increase in hepatic blood flow, which suggests a reduction in the hepatic vascular resistance (163,167). However, long-term prazosin administration is associated with a significant reduction in arterial pressure and systemic vascular resistance and an activation of endogenous vasoactive systems that lead to plasma volume expansion, sodium retention, and, in some cases, ascites. Recent data suggest that the adverse effects of prazosin on the systemic circulation and renal function may be attenuated by the combined administration of prazosin and propranolol. In a randomized controlled investigation, the combination of propranolol and prazosin was significantly more effective, in terms of reducing the HVPG, than the combination of propranolol and IMN, although it was less well tolerated (168). However, this drug combination has not been assessed in long-term RCTs of the prevention of variceal hemorrhage.

Clonidine is a centrally acting α_2-adrenergic agonist that reduces peripheral adrenergic output. Its hemodynamic effects are therefore similar to those of both β- and α-adrenergic blockers. In the systemic circulation, clonidine reduces the heart rate, cardiac index, and arterial blood pressure, whereas in the splanchnic circulation, it reduces the HVPG by decreasing portal and hepatic resistance and splanchnic inflow (169). Despite the marked fall in arterial pressure after clonidine administration, hepatic blood flow and liver and renal function are maintained. The fall in the HVPG is slightly greater than that achieved with propranolol.

Carvedilol is a nonselective β-adrenergic blocker with intrinsic anti–α-adrenergic activity, which indicates that its effects are similar to those of clonidine without some of its inconveniences (rebound adrenergic hyperactivity) (170).

Blockade of the renin–angiotensin system

Activation of the renin–angiotensin system is a frequent finding in patients with cirrhosis, especially those with more advanced disease. This is thought to represent a homeostatic response to counterbalance the systemic and splanchnic vasodilatation and arterial hypotension observed in portal hypertension. A direct relation between HVPG and plasma renin activity has been observed in patients with cirrhosis (171), and several studies have demonstrated that the infusion of angiotensin II increases portal pressure by increasing the hepatic vascular resistance. These observations provided the rationale of renin–angiotensin blockade in portal hypertension. Initial studies with the intravenous administration of *saralasin*, a peptide-competitive antagonist of angiotensin II, showed a reduction in the HVPG but also a marked reduction in arterial pressure (172). Angiotensin-converting enzyme inhibitors have also been tested. Although no effect on HVPG was observed with *captopril*, a significant reduction was observed with *enalapril* in two studies performed in a small group of patients. However, a reduction in arterial pressure was frequently noted in these studies. More recently, in a nonrandomized study of patients with portal hypertension, *losartan*, a nonpeptide antagonist of angiotensin

receptor type I, was reported to markedly reduce portal pressure (173). However, these results were not reproduced in subsequent studies, in which significant reduction in arterial pressure and almost negligible effects on HVPG were observed (174).

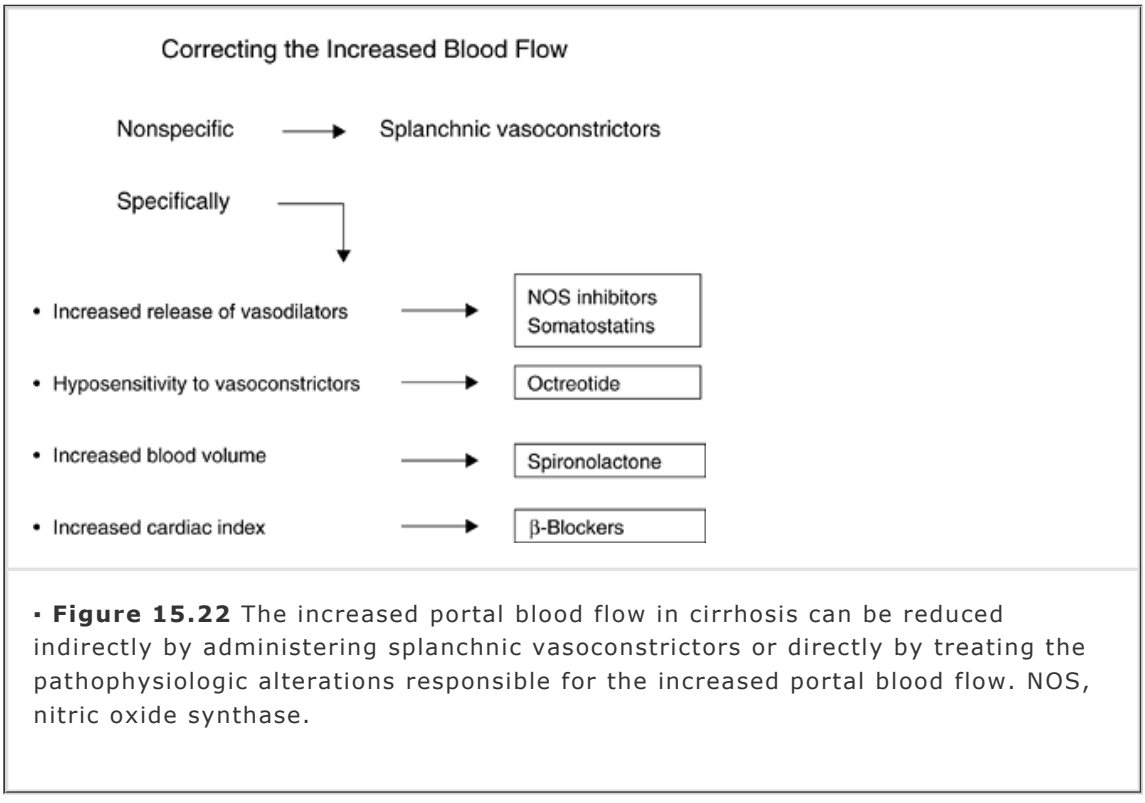
However, these agents may still have a role in preventing the progression of hepatic fibrosis in early stages of the disease, as suggested by studies in cirrhotic rat models (175).

Blockade of endothelin

Conflicting results have been obtained with ET blockers in experimental models of portal hypertension. Acute administration of the mixed ET-A and ET-B receptor blocker bosentan decreased portal pressure (32), whereas long-term administration of Ro 48-5695, a second-generation mixed ET receptor blocker, did not modify portal pressure and even increased liver fibrosis (34). Long-term selective blockade of ET-A receptor with LU 135252 dramatically decreased collagen accumulation in a model of secondary biliary cirrhosis (176), whereas the acute administration of another ET-A blocker (FR 139317) to cirrhotic rats did not lower portal pressure (177). It seems obvious that the pathophysiologic role of ET must be further clarified.

Calcium channel blockers

Experimental studies in isolated perfused liver suggest that calcium channel blockers may decrease portal pressure in cirrhosis by reducing the hepatic vascular resistance. However, several studies in patients with cirrhosis in which verapamil, nifedipine, and nicardipine were tested failed to show any reduction in HVPG. Nicardipine improved hepatic perfusion without modifying HVPG but increased the portal–collateral blood flow, an effect that might be dangerous in patients with esophageal varices. Overall, these data indicate that calcium channel blockers have no role in the treatment of portal hypertension (5).



▪ **Figure 15.22** The increased portal blood flow in cirrhosis can be reduced indirectly by administering splanchnic vasoconstrictors or directly by treating the pathophysiologic alterations responsible for the increased portal blood flow. NOS, nitric oxide synthase.

Pharmacologic Agents that Decrease Splanchnic Blood Flow

Most pharmacologic treatments of portal hypertension have attempted to correct the increase in portal blood inflow with splanchnic vasoconstrictors (Fig. 15.22). Some of these drugs, such as nonselective β-blockers, can be given orally and are therefore

appropriate for the long-term treatment of portal hypertension, whereas others, such as somatostatin or terlipressin, require parenteral administration and are used only on a short-term basis, as in the treatment of acute variceal hemorrhage.

Nonselective β-blockers

The reduction in portal venous inflow by nonselective β-blockers such as propranolol, nadolol, and timolol is the consequence of a decrease in cardiac output caused by blockade of cardiac β_1-adrenoceptors and of splanchnic vasoconstriction caused by blockade of vasodilating β_2-adrenoceptors in the splanchnic vasculature (178,179,180). This dual effect explains why cardioselective β_1-blockers reduce the portal pressure less effectively than nonselective β-blockers. The reduction in portal pressure averages approximately 14% and is accompanied by a significant reduction in esophageal variceal pressure and azygos blood flow, which is an index of blood flow through the gastroesophageal collaterals and esophageal varices. The

mean reduction in azygos blood flow with propranolol is approximately 35% (109). The significant effect of propranolol on portal pressure and the pronounced reduction in collateral blood flow explain why this drug reduces both variceal pressure (which decreases in parallel with portal pressure) *and* the volume/radius of the varices (which are reduced in the same proportion as azygos blood flow). Because of these two effects, propranolol causes a greater decrease in variceal wall tension than in variceal pressure (134).

β-Blockers are contraindicated in approximately 15% of patients who are considered for this treatment. Side effects are reported in approximately 15% to 20%, but severe events are rare. The most frequent complaints are fatigue, shortness of breath (often associated with marked bradycardia), and sleep disorders. Some of these side effects disappear with time or after the dose has been reduced. Although the complications of propranolol therapy in cirrhosis have never been lethal, side effects are important because they discourage compliance.

Nadolol is easier to administer because it has a longer half-life (allowing once-a-day administration) and is eliminated by the kidneys. Also, it has been suggested that, because it does not cross the blood–brain barrier, it is less likely to cause central effects. Frequently, side effects become less severe after propranolol is exchanged for nadolol, or vice versa.

A more important limitation of nonselective β-blockers is that they achieve the targeted reduction in HVPG in only about one third of patients with advanced cirrhosis (143). This limitation is, at least in part, a consequence of the fact that these drugs increase the portal collateral resistance, which attenuates the fall in portal pressure that should be expected from the reduction in portal–collateral blood flow. This explains why patients with advanced portal hypertension, who have already bled from varices, do not respond as well as compensated patients or patients without varices (178,181).

Vasopressin and analogs

Vasopressin is thought to be the most powerful vasoconstrictor; however, its clinical efficacy is still controversial. At pharmacologic doses, it reduces the blood flow to all splanchnic organs and thereby decreases portal blood flow and portal pressure. Vasopressin is also effective in reducing collateral blood flow and variceal pressure. Adverse hemodynamic effects of vasopressin are systemic vasoconstriction with increased peripheral vascular resistance and reduced cardiac output, heart rate, and coronary blood flow. These effects may result in serious complications, such as myocardial ischemia or infarction, arrhythmias, mesenteric ischemia, limb ischemia, and cerebrovascular accidents. In 25% of cases, vasopressin therapy must be

withdrawn because of such complications (182).

The *combination of NTG and vasopressin* enhances the reduction in portal pressure and attenuates the systemic side effects of vasopressin. NTG, which acts by delivering NO, reduces the vascular resistance in the liver microcirculation and portal venous system and improves myocardial performance (183). Several RCTs and meta-analyses have shown that this drug combination is more effective and safer than vasopressin alone in controlling bleeding from ruptured esophageal varices. Therefore, if vasopressin is used, it should always be combined with NTG. However, better pharmacologic alternatives are now available, such as terlipressin and somatostatin.

Terlipressin is a synthetic analog of vasopressin (triglycyl-lysine vasopressin). After intravenous injection, it is slowly converted into vasopressin through enzymatic cleavage of the triglycyl residues by tissue peptidases; therefore, tissue levels are high and circulating levels low. Terlipressin, unlike vasopressin, does not enhance fibrinolysis and its biologic activity lasts longer, so that continuous intravenous infusion is unnecessary. The preferred schedule of administration is intravenous injection of 2 mg every 4 to 6 hours (184). Terlipressin is not readily available in the United States.

Somatostatin and analogs

Somatostatin was introduced for the treatment of variceal hemorrhage because it decreases portal pressure without the adverse effects caused by vasopressin on the systemic circulation. Somatostatin causes splanchnic vasoconstriction and thereby decreases portal and collateral blood flow and portal pressure (185). Somatostatin probably inhibits the release of splanchnic vasodilator peptides, such as glucagon, and enhances the effects of endogenous vasoconstrictor systems. In addition, somatostatin blocks postprandial increases in portal blood flow and portal pressure. Bolus injections of somatostatin cause marked vasoconstriction in humans, characterized by rapid and intense, but transient, falls in portal pressure and azygos blood flow; the effects of bolus injections are much more pronounced than those of continuous infusions (186). Usually, somatostatin is administered as an intravenous bolus injection of 250 µg, which is followed by continuous infusion at a rate of 250 µg/hour for 2 to 5 days. However, the dosage used for continuous infusions is largely empiric because hemodynamic studies have shown that to achieve a consistent effect, with a reduction of both HVPG and azygos blood flow, a dosage of 500 µg/hour is required (186). Actually, better clinical results are achieved by the 500 µg/hour infusion dosage (187).

Octreotide, vapreotide, and *lanreotide* are cyclic octapeptide analogs of somatostatin that have a longer biologic half-life. Somatostatin analogs have been shown to reduce portal pressure in animals; however, their hemodynamic effects in patients with cirrhosis are controversial (185). Octreotide injection is followed by rapid desensitization and a loss of its effects in decreasing HVPG and azygos blood flow and suppressing circulating glucagon levels. However, the effects of octreotide in attenuating postprandial splanchnic hyperemia may be maintained (188).

Nitric oxide synthase inhibitors

Blocking NO synthesis in animals with experimental cirrhosis has been shown to correct the hyperdynamic circulation (66,67). In a recent report, the systemic administration of the NO synthase inhibitor *N*(G)-monomethyl-L-arginine to patients with compensated cirrhosis and portal hypertension decreased the hyperkinetic systemic circulation and enhanced renal function and sodium excretion (189). However, the study did not assess its effects on the HVPG. In experimental models, the reduction of portal blood flow by NO blockade was not paralleled by a decrease in portal pressure because of a concomitant increase in hepatic and collateral resistance. Therefore, there is no place

for the use of NO blockers in reducing splanchnic blood flow and portal pressure.

Other Drugs and Drug Combinations

It is unlikely that any single agent can decrease the portal pressure sufficiently to provide complete protection from the risk of variceal bleeding or rebleeding in most patients. Prevention is probably more easily achieved by using combinations of drugs that act through different mechanisms. The strategy for the treatment of portal hypertension may profit from the experience gained in the treatment of systemic hypertension.

Drug combinations

The first drug combination used in patients with portal hypertension was *vasopressin and NTG* to treat acute variceal bleeding. The same concept (combination of a vasoconstrictor and a nitrovasodilator) was thereafter applied by administering *propranolol and IMN* (190). This drug combination has been repeatedly shown to cause a greater reduction in portal pressure than either drug alone, so that "good" or "optimal" responses (decreases in HVPG of at least 20% of baseline values or to below 12 mm Hg) are achieved in a greater number of patients. Part of the reason for the efficacy of this combination is that IMN prevents the increase in portal resistance caused by propranolol. It is important to emphasize that unlike the acute administration of IMN, long-term treatment with the combination of propranolol (or nadolol) and IMN does not cause deleterious effects on renal function or sodium handling in patients with cirrhosis (162). This result is different from what occurs when propranolol and ISDN are combined. Adding IMN achieves the target decrease in HVPG in one third of propranolol nonresponders (a la carte treatment) (191).

Other drug combinations have been shown to enhance the portal pressure–reducing effect of propranolol, either in experimental models of portal hypertension or in patients with liver cirrhosis. These include propranolol and serotonin antagonists, propranolol and spironolactone, propranolol and clonidine, and *propranolol and prazosin*. This last combination is the most interesting one because its portal pressure–reducing effect is significantly greater than that of propranolol plus IMN (168). It should be emphasized that long-term combination therapy enhances the fall in portal pressure only in those patients who fail to exhibit an adequate response to propranolol or nadolol alone. In other words, patients who "respond" to β-blockers do not benefit from the addition of a vasodilator.

Drugs with combined actions

Nipradilol combines in one molecule the action of a nonselective β-blocker and a nitrate-like vasodilator activity. Studies in experimental models suggest that nipradilol may achieve a greater reduction in portal pressure than propranolol (192). However, this finding has not been confirmed in patients with liver cirrhosis (193).

Carvedilol, a nonselective β-blocker with intrinsic α_1-adrenergic antagonist activity, has been used to treat arterial hypertension, ischemic heart disease, and heart failure. Its effects in HVPG are greater than those of propranolol (see preceding text) (170).

Other Pharmacologic Approaches

Manipulation of splanchnic blood volume

Peripheral vasodilatation in cirrhosis plays a major role in the activation of endogenous neurohumoral systems, which in turn leads to sodium retention and expansion of the plasma volume. Expansion of the plasma volume is thought to worsen portal hypertension by increasing the cardiac index. Indeed, the administration of *spironolactone* and a low-sodium diet have both been shown to reduce portal pressure

in patients with cirrhosis. Reduction of the plasma volume may trigger vasoactive mechanisms that further decrease the splanchnic blood flow and portal pressure (194). Clearly, the concomitant use of diuretics by patients with portal hypertensive cirrhosis may be an important factor affecting portal

pressure that has not been taken into account sufficiently in the past and that may explain in part some conflicting results of previous clinical trials.

Drugs that increase pressure in the lower esophageal sphincter

It has been suggested that metoclopramide, domperidone, and other drugs that increase pressure in the lower esophageal sphincter be used as ancillary therapy in the management of variceal bleeding, on the basis of the finding that they reduce the inflow of blood into varices in portographic studies and significantly decrease azygos blood flow and variceal pressure. However, their value in the management of variceal bleeding is uncertain.

Noninvasive Evaluation of Response to Drug Therapy

The wide variations in HVPG response of individual patients to pharmacologic treatment suggests that it would be desirable to schedule follow-up measurements of the HVPG during long-term pharmacologic therapy, to determine whether the treatment is likely to offer adequate protection from the risk for bleeding. Although measurement of the HVPG is a simple and safe technique, it is an invasive investigation. For this reason, substantial interest has been shown in developing noninvasive methods to monitor the hemodynamic response to pharmacologic therapy. These methods are summarized in the subsequent text.

Duplex Doppler ultrasonography

Initial studies evaluated the use of Doppler ultrasonography to measure changes in the femoral blood flow, after the acute administration of propranolol, in the hope that these would correlate with the splanchnic hemodynamic effects. However, the test identified only a fraction of patients with a poor HVPG response to propranolol and none of those with a good response. Therefore, when a reduction in femoral blood flow was absent or minimal, HVPG response was usually poor, but only about half the patients with a marked reduction in femoral blood flow had a clinically significant reduction in portal pressure (50% positive predictive value) (195). Similar results were found when changes in forearm blood flow were measured by strain gauge plethysmography (196).

The duplex Doppler technique has been used to assess changes in portal blood flow and blood flow velocity and changes in the superior mesenteric artery blood flow velocity and pulsatility index after β-blockade. Although it has been suggested that the overall predictive accuracy of duplex Doppler in identifying "good" and "bad" responders to nonselective β-blockers may be high, these results have not been confirmed, probably because the intraobserver and interobserver variability of duplex Doppler measurements is relatively high (197,198). On the other hand, most studies evaluating duplex Doppler for predicting the HVPG response have used β-blockers. However, this technique is not adequate to assess changes in portal pressure caused by drugs or drug combinations that modify vascular resistance.

Measurements of variceal pressure

Several studies have shown that the noninvasive measurement of variceal pressure with a pressure-sensitive endoscopic gauge may be of value in assessing the hemodynamic response to β-blockers and the risk for variceal bleeding during follow-up. In a longitudinal, prospective study, a fall in variceal pressure by more than 20%

of baseline values was associated with a very low risk for variceal bleeding on follow-up (5% probability of variceal bleeding on long-term follow-up vs. 41% in patients failing to achieve such a marked response), which suggests that variceal pressure measurements may provide useful prognostic information (117) (Fig. 15.23). However, the measurement of variceal pressure is of limited applicability, mainly because measurements are frequently not possible in small varices, and artifacts caused by esophageal peristalsis may prevent satisfactory measurements. However, measurements of variceal pressure have the potential advantage of being applicable in patients with esophageal varices of any cause, including prehepatic portal hypertension, in which measurement of the HVPG is not adequate to assess the portal pressure.

Interventional Treatment of Acute Bleeding Related to Portal Hypertension

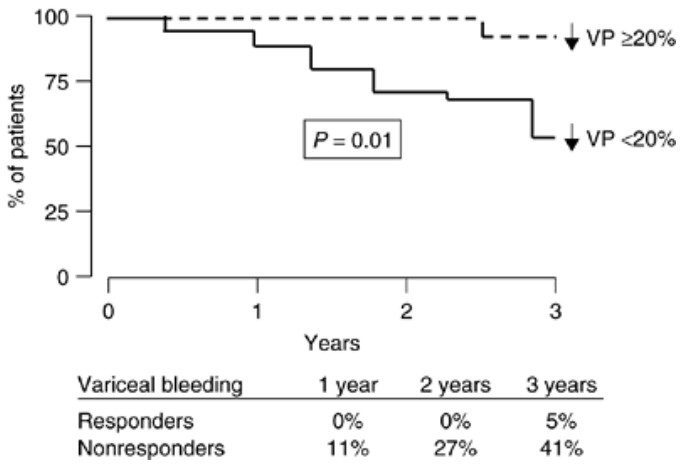
Endoscopic therapy

The endoscopic procedures for managing bleeding esophageal varices are described later in this chapter.

Balloon tamponade

The Sengstaken-Blakemore tube is a triple-lumen tube with gastric and esophageal balloons and a tube for aspirating the stomach. The Minnesota tube has a larger gastric balloon and a lumen for aspirating the esophagus. The Linton-Nachlas tube, which is used for gastric varices, has a large gastric balloon and two lumina for aspirating the stomach and esophagus. Balloon tamponade may temporarily control bleeding in 60% to 90% of patients. On deflation of the balloons, bleeding recurs in approximately 50% of cases (199). Balloon tamponade is used only for 12 to

24 hours in emergency situations as a bridge to definitive therapy if drugs and endoscopy fail to control variceal bleeding. Complications occur in 10% to 15% of patients and mainly involve aspiration pneumonia and, rarely, esophageal rupture. Complication-related mortality ranges between 2% and 5%. Only well-trained personnel should attempt tamponade (199).



Variceal bleeding	1 year	2 years	3 years
Responders	0%	0%	5%
Nonresponders	11%	27%	41%

▪ **Figure 15.23** Prognostic value of repeated measurements of variceal pressure (VP) with an endoscopic pressure gauge (Varipress) in patients on pharmacologic therapy. A fall in VP of more than 20% of the baseline values is associated with a much higher actuarial probability of the patient remaining free of bleeding at follow-up (5% probability of variceal bleeding vs. 41% in patients failing to

achieve such a marked response, *P* = 0.01). (From Escorsell A, Bordas JM, Castaneda B, et al. Predictive value of the variceal pressure response to continued pharmacological therapy in patients with cirrhosis and portal hypertension. *Hepatology* 2000;31:1061–1067, with permission.)

Percutaneous transhepatic embolization of gastroesophageal varices

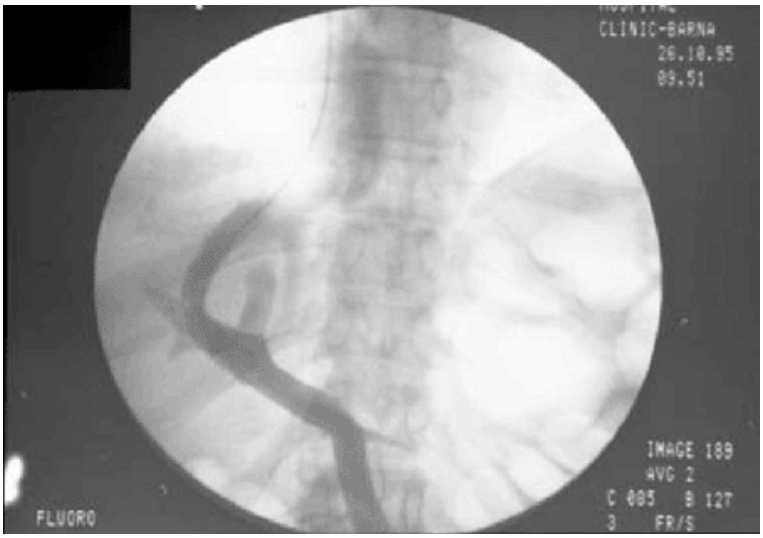
Percutaneous transhepatic embolization of gastroesophageal varices, first proposed in the early 1970s, was practically abandoned after it was shown to be less effective than sclerotherapy (200). It is performed by catheterizing the gastric collaterals that supply blood to varices through the transhepatic route. Percutaneous transhepatic embolization is used nowadays only as a complement to TIPS in patients with severe acute bleeding, especially in patients with gastric varices.

Portosystemic shunts

Transjugular intrahepatic portosystemic shunt

TIPS is an intrahepatic calibrated portosystemic shunt performed between an intrahepatic branch of the portal vein (usually the right) and one hepatic vein (usually the right) using vascular intervention radiologic techniques (Fig. 15.24). Immediately after TIPS placement, there is a marked reduction in the PPG. This is due both to a marked decrease in absolute portal pressure and a slight increase in the IVC pressure. Reduction in the PPG after TIPS is also associated with a marked reduction in portal–collateral blood flow. TIPS is very effective in variceal bleeding refractory to medical and endoscopic treatments. Indeed, complete protection from variceal bleeding is achieved when PPG decreases below 12 mm Hg (1). Therefore, the goal of TIPS is to reduce the PPG below the threshold gradient of 12 mm Hg. Despite the marked reduction in PPG observed after TIPS, PPG tends to increase again during follow-up because of the development of stenosis or occlusion of the shunt in the stent, at the portal vein opening, or, more frequently, at the hepatic vein. Progression of the stenosis will again promote the increases in PPG above the 12 mm Hg threshold (TIPS dysfunction) and patients reenter the bleeding risk zone. The actuarial probability of TIPS dysfunction with the use of bare stents is greater than 75% after 1 year of follow-up. The introduction of the expanded

polytetrafluoroethylene (ePTFE)-covered stents has completely changed this scenario, with a reported 1-year actual rate of dysfunction as low as 10% (201).



▪ **Figure 15.24** Transjugular intrahepatic portosystemic shunt. A calibrated shunt between the portal vein and the inferior vena cava.

Surgical procedures are discussed in Chapter 16.

Prevention of the First Bleed from Esophageal Varices

Pharmacologic treatment

Pharmacologic prophylaxis is aimed at preventing the first bleeding episode and at improving survival by reducing bleeding-related deaths. More recently the hypothesis that drug therapy may prevent development or progression of varices has been tested (*preprimary prophylaxis*) (202). Nonselective β-blockers, nitrates, and spironolactone are the only drugs assessed for primary prophylaxis of variceal bleeding. Although several risk factors for variceal bleeding have been identified, clinical trials assessing pharmacologic treatments for prophylaxis of first bleeding included patients mainly on the basis of variceal size.

Nonselective β-blockers

As discussed in a preceding section in this chapter, nonselective β-blockers reduce portal pressure through a reduction in portal and collateral blood flow.

Three RCTs assessed nonselective β-blockers for preprimary prophylaxis with discordant results (85,203,204). Two of these studies included patients with small varices (although 79/206 patients had no varices in one study) (204). In one study (204) an unfavorable effect of propranolol was found, while in the other (203) nadolol effectively reduced the progression of varices and the bleeding risk. The discordant results may be explained, at least in part, by the high proportion of dropouts (almost one third) in the first study (203). The third, the only study that was double blind, aimed at assessing whether timolol prevents the development of varices in patients with HVPG greater than 6 mm Hg but failed to show a beneficial treatment effect, although it was confirmed that patients in whom HVPG was below 10 mm Hg or in whom HVPG decreased by greater than 10% had a significantly lower risk of developing varices. Therefore, at present, the conclusion for preprimary prophylaxis is that there is no indication to treat patients without varices for the prevention of the formation of varices; patients with small varices could be treated with a nonselective β-blocker, but

further studies are required before a formal recommendation may be made (8).

The efficacy of the nonselective β-blockers propranolol and nadolol for the prophylaxis of the first variceal bleeding has been compared with that of placebo or nonactive treatment in 13 RCTs (141,205), including mainly patients with medium or large esophageal varices. Several meta-analyses of these studies consistently showed a significant reduction in the risk for bleeding, from 25% with nonactive treatment to 15% with β-blockers (135) over a median follow-up of nearly 2 years (135,141,205,206). According to this bleeding risk reduction, one bleeding episode will be prevented in every 10 treated patients. Mortality was only slightly reduced from 27% to 23%—this effect barely approached the level of statistical significance. The lack of a significant reduction of mortality with β-blockers is probably due to inadequate power of the reported studies and to their relatively short follow-up.

Overall, available data, including subgroup analyses (85,135,141,203,204,205,206), shows that nonselective β-blockers are more beneficial in patients with medium or large varices without ascites than in those with ascites. The effect is small and nonsignificant in patients with small varices.

Several contraindications remarkably reduce the number of patients suitable for β-blocker therapy. The most frequent are active pulmonary obstructive disease, aortic valve disease and other heart diseases that may be worsened by β-blockers, atrioventricular heart block, and peripheral arterial insufficiency. Sinus bradycardia and insulin-dependent diabetes are relative contraindications. Because of these contraindications, approximately 15% of patients are excluded from therapy with β-blockers in clinical practice (207). The incidence of side effects among treated patients is approximately 15%. The most frequent are fatigue, dyspnea, bronchospasm, and reduction of sexual activity. Five percent of side effects require treatment discontinuation. Renal function is not affected. Hepatic encephalopathy has been attributed to β-blockers in rare patients with severe liver disease. However, in patients without severe hepatocellular dysfunction or history of encephalopathy, propranolol does not impair cerebral blood flow, arterial ammonia, or neuropsychologic functions.

Propranolol should be initially dosed at 20 mg b.i.d. and titrated on a biweekly basis, up to a maximum of 160 mg b.i.d. In most clinical trials the dosage has been titrated empirically to decrease the resting heart rate by 25% of baseline value. However, this target is not related to the hemodynamic effect of the drug. A reduction of HVPG to levels less than 12 mm Hg was found to abolish the risk of variceal bleeding (131,208). However, the efficiency of such a hemodynamic target in clinical practice is still uncertain in patients treated for prevention of first bleeding who have a relatively low bleeding risk. Therefore, in clinical practice propranolol is empirically dosed up to the maximum tolerated dose, within the limit of 160 mg b.i.d. This results in a median dose of near 80 mg b.i.d. Similar concepts apply to nadolol, which should be dosed at 20 mg a day in a single daily dose and titrated every 2 to 3 days up to a

maximum of 160 mg; the median maximum tolerated daily dose is 80 mg. When propranolol is withdrawn, the risk of variceal hemorrhage returns to what would be expected in an untreated population, and the risk of mortality is higher than that observed in an untreated population. These observations support the current practice of indefinite prophylactic therapy (209).

After cessation of propranolol therapy bleeding may occur in some patients; therefore, patients should be instructed not to discontinue treatment, and if needed, to be under direct monitoring by a physician.

Organic nitrates

Administration of short-acting (e.g., NTG) or long-acting (e.g., ISDN or IMN) nitrates decreases portal pressure mainly through a reduction in intrahepatic and portal–

collateral resistance (5). As discussed in another section of this chapter, vasodilators may adversely affect renal function and are not used as single agents for the treatment of portal hypertension (5,210). However, the combination of a vasodilator (e.g., IMN) with a vasoconstrictor (e.g., propranolol or nadolol) enhances the fall in portal pressure achieved by either drug alone while preventing its systemic side effects (162,167,168,190). These combinations achieve a greater reduction of portal pressure and also allow a significant additional portal pressure reduction in some of the patients who are *nonresponders* to propranolol.

Among long-acting nitrates, only IMN has been evaluated in clinical trials for the prevention of variceal bleeding. It has been compared with propranolol in three studies including 276 patients overall. Pooled results from these three RCTs showed a nonsignificant increase in bleeding rate and mortality with IMN (141). One study of IMN versus β-blockers reported an increased mortality with IMN among patients older than 50 (141). Although this may reflect an improved prognosis in the group on β-blockers, it suggests that IMN should not be used as single agent. Moreover, a recent double-blind study of IMN versus placebo in patients with contraindications or intolerance to β-blockers failed to show any benefit from IMN in the prevention of first bleeding (207).

The combination of IMN and β-blockers has been compared with β-blockers alone in three RCTs (211,212,213). Two studies used nadolol and one propranolol. The first one, an open study, showed a significant bleeding risk reduction with combination therapy, without significant effects on mortality (211). The other two studies were double blind and placebo controlled. One was a small single-center study including 57 patients with large varices and red color sign and failed to show any significant advantage with the combination therapy, although it was clearly underpowered (212). The last one, a multicenter study, included 349 patients (199 with large varices) and found no differences between the two treatment groups in bleeding or mortality rates, even when only patients with large varices were considered (213). Overall, 552 patients were included in the three studies. Bleeding rate was 15% in the β-blocker–treated patients and 10% in the combination therapy patients; the 5% bleeding risk reduction with the combination therapy was not significant (95% CI -16% to 6%). Mortality rate was 10% with both treatments, and side effects were significantly more frequent with combination therapy. No evidence of impairment of renal function or of worsening or increased incidence of ascites was found with nitrates in any of these studies (141). These results do not support the use of combination therapy for the prevention of first variceal bleeding.

Major side effects of nitrates are headache (which occurs in nearly 20% of patients but rarely requires treatment discontinuation) and hypotension (63,207). When nitrates are given in combination with β-blockers, side effects occur in 30% to 50% of patients and leads to discontinuation of nitrates in 10% to 40% of patients. The probability of side effects with nitrates is dose dependent. If the choice to give nitrates to a patient is made, then IMN should be started at a dosage of 10 mg b.i.d. and titrated every other day up to 40 mg b.i.d. A dosage of 10 to 20 mg b.i.d. may be better tolerated and cause less systemic side effects, while being able to cause intrahepatic and collateral vasodilatation (46).

Diuretics

Spironolactone, by counteracting the increase in plasma volume that sustains the hyperdynamic circulation in portal hypertension, lowers HVPG in patients with cirrhosis (194). Its combination with nadolol has been compared with nadolol alone for the prevention of first variceal bleeding and ascites in compensated patients with cirrhosis in an RCT including 100 patients (214); similar HVPG reductions (-16% ± 12% vs. -11% ± 14%), bleeding rates (17% vs. 14%), and 2-year mortality rates (2% vs. 6%) were achieved in both groups.

Monitoring pharmacologic treatment response

In prospective cohort studies and clinical trials it has been shown that the risk of bleeding is virtually abolished when HVPG or the variceal pressure is reduced by as much as or more than 20% of baseline values or HVPG is reduced below 12 mm Hg (117,131,143,215,216,217). Overall, the proportion of patients in whom HVPG was reduced to 12 mm Hg or less was 21%; in these patients the odds ratio for bleeding compared to those who did not reach this hemodynamic target was 0.21 (95% CI 0.05 to

0.80). The proportion of patients with HVPG reduction by as much as or more than 20% of baseline values or to 12 mm Hg or less was 31%, and the odds ratio for bleeding in these patients compared to those not reaching this target was 0.14 (95% CI 0.05 to 0.42). However, failure to reach a treatment response according to these hemodynamic targets does not mean that the patient will bleed. In fact, approximately 60% of patients who do not achieve these targets after being treated prophylactically with β-blockers will not bleed. Because of this relatively low bleeding risk, there is consensus that hemodynamic monitoring of pharmacologic treatment response is not a definite indication during the prophylaxis of the first variceal bleeding (8).

Endoscopic Therapy

Endoscopic injection sclerotherapy

In endoscopic injection sclerotherapy, a substance that induces thrombosis in the vessel and inflammation of the surrounding tissue is injected into the variceal lumen (intravariceal technique) or adjacent to the varix (paravariceal technique) to obliterate the varix. The injection needle is passed through a flexible endoscope. Injections are initiated at or just above the gastroesophageal junction and proceed upward. About 1 to 3 mL of sclerosant is injected at each site; the risk for complications increases as the volume of sclerosant injected increases. Variceal obliteration is usually achieved after three to six sclerotherapy sessions. After variceal obliteration, follow-up endoscopic procedures are carried out at 3-month intervals for the first 6 months and at 12-month intervals thereafter to detect and treat recurrent varices.

The most widely used sclerosing agents are 1% to 3% polidocanol, 5% ethanolamine oleate, 1% to 2% sodium tetradecyl sulfate, and 5% sodium morrhuate. No significant differences have been shown between sclerosants, in terms of either efficacy or complications.

Complications of sclerotherapy are relatively frequent and sometimes severe enough to require treatment discontinuation (218). Minor complications such as low-grade fever, retrosternal pain, transient dysphagia, and asymptomatic pleural effusions are common, occur within the first 24 to 48 hours, and do not require treatment. Asymptomatic transient bacteremia has been reported after treatment in 30% to 50% of cases. Esophageal ulcers occur in up to 90% of patients within 24 to 48 hours of injection and heal rapidly in most cases. The ulcers that follow sclerotherapy are frequently asymptomatic but may precipitate bleeding in up to 20% of patients. Esophageal ulcers hamper further injections, delay successful variceal obliteration, and prolong the risk for bleeding that is associated with varices. Sucralfate and omeprazole have been suggested as being potentially useful in healing esophageal ulcers, but convincing proof of such an effect is not available. Esophageal stenosis may occur in 2% to 10% of patients and frequently requires dilatation. Full-thickness esophageal wall necrosis resulting in esophageal perforation is rare but almost always fatal. PVT, seizures, and sepsis have also been reported. Altogether, the procedure-related mortality is approximately 1% in elective cases (218). Morbidity and mortality are higher when the procedure is performed on an emergent basis in actively bleeding

patients.

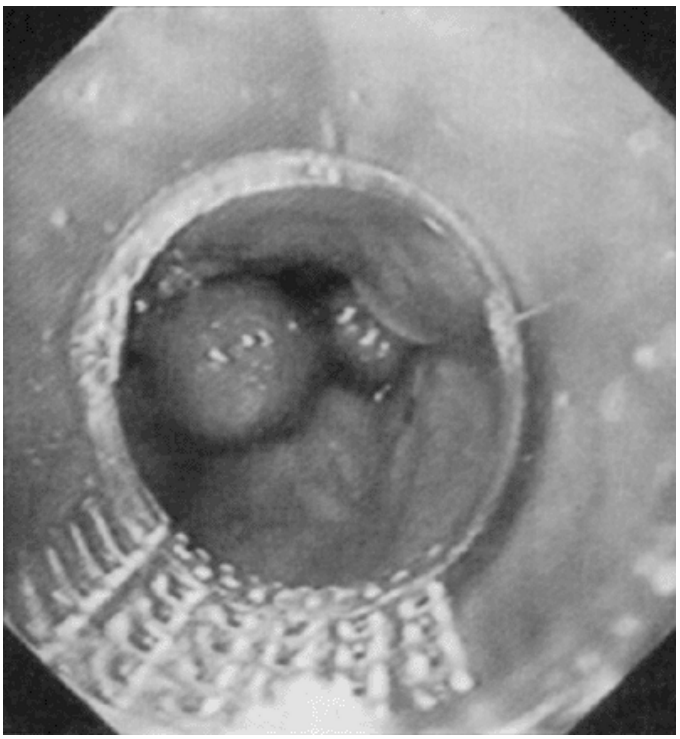
Variceal obturation

The injection of tissue adhesives such as *N*-butyl-2-cyanoacrylate (Histoacryl) and isobutyl-2-cyanoacrylate (Bucrylate) have been used for both esophageal and gastric varices (218). The adhesives polymerize within seconds of injection, forming a solid cast that obliterates the injected vessel. After 2 to 3 weeks, the overlying mucosa sloughs off, and a glue cast is extruded into the lumen of the gastrointestinal tract. The ensuing ulceration, which may be extensive and deep, usually heals rapidly. Pulmonary embolism and cerebrovascular accidents resulting from dissemination of the tissue adhesive into the systemic circulation have been reported.

Endoscopic banding ligation

In banding ligation, rubber rings are placed on the varices, which are sucked into a hollow cylinder attached to the tip of an endoscope (Fig. 15.25). The blood flow is completely interrupted; ischemic necrosis

of the mucosa and submucosa follows, and granulation takes place thereafter, with sloughing of the rubber rings and necrotic tissue. The whole process leaves shallow mucosal ulcerations that heal in 14 to 21 days (219,220). Multiple-shot devices allowing the placement of four to ten bands at a time have made the technique easier to perform than was possible with the one-shot devices previously available. Application of the bands is started at the gastroesophageal junction and progresses upward in a helical arrangement for approximately 5 to 8 cm until four to eight bands have been applied. Banding ligation sessions are repeated at 14-day intervals until the varices are obliterated, which usually requires two to four ligation sessions.



▪ **Figure 15.25** Esophageal band ligation. Variceal nodule ligated with an elastic band. (Courtesy of Drs. J. M. Bordas and J. Llach.)

As with sclerotherapy, minor complications such as transient dysphagia and chest discomfort are relatively frequent. The shallow ulcers that develop at the site of ligation bleed less frequently than the ulcers that form after sclerotherapy (219,220). Mechanical complications that follow use of the overtube (formerly required when only one-shot devices were available), which range from mucosal tears that cause bleeding to complete esophageal perforation, are less frequent now that multiple-band ligating devices are coming into widespread use (218).

Detachable miniloops

Detachable nylon miniloops have been tested as an alternative to endoscopic band ligation to treat both esophageal and gastric varices. The technique is similar to band ligation. A varix is sucked into the cylinder, and a miniloop (similar to a polypectomy snare) is passed through the working channel of the endoscope, tightened around the entrapped varix, and detached from its shaft. The procedure can be repeated several times, and multiple varices can therefore be ligated with a single insertion of the endoscope. The initial results of the procedure are promising (221), but the technique must be compared with multiple ligation devices in a much larger number of patients.

Both endoscopic injection sclerotherapy and banding ligation have been used to prevent first variceal bleeding, mostly with unconvincing results.

Endoscopic treatment

Endoscopic injection sclerotherapy has been compared with no treatment in 22 RCTs including 2,052 patients (141,218). The results of these studies are significantly heterogeneous because of marked differences in the baseline bleeding risk (the risk of untreated controls). Sclerotherapy significantly reduced the risk of bleeding in RCTs with baseline bleeding risk over 40%, had no effect in RCTs with baseline risk between 20% and 40%, and was harmful with baseline risk below 20% (Fig. 15.26). Because no criteria are available in clinical practice to accurately select patients at such a high risk as to benefit from prophylactic sclerotherapy, there is a wide consensus (118) that sclerotherapy should not be used for the prevention of first variceal bleeding.

In two of these 22 studies a three-arm design (placebo or no treatment vs. sclerotherapy vs. propranolol) was used. A total of 226 patients were included in the arms comparing sclerotherapy with propranolol. Patients were not selected for variceal size. No significant differences were found in the risk of variceal bleeding, but in one of these studies, a significant difference in favor of propranolol was found regarding bleeding from any portal hypertensive source.

The combination of sclerotherapy with propranolol has been found to be less effective than propranolol alone in one RCT (141).

The efficacy of *endoscopic banding ligation* compared with no treatment has been assessed in six RCTs (three still available only in abstract) including 675 patients (222,223). Although these studies were questionable from an ethical point of view because controls were left without treatment when it was already known that nonselective β-blockers significantly reduce the risk of bleeding in such cases, the studies showed that banding ligation significantly reduces both bleeding and risk of death. However, it is notable that the only trial assessing banding ligation in patients who could not tolerate β-blockers (224) failed to show any benefit from banding.

Banding ligation was then compared to β-blockers in 12 RCTs (222). Overall, the results show a marginal benefit from banding ligation in terms of bleeding, but not in mortality. Of note, only seven studies (including only 574 patients) have been published as full reports (225,226,227,228,229,230,231). A meta-analysis of these seven studies (222) showed a nonsignificant trend toward a bleeding risk reduction with ligation (relative risk [RR] 0.66, 95% CI 0.44 to 1.01). The only study showing a significant benefit with ligation (225) used a dosage of propranolol remarkably lower

than the median dose of previous RCTs on prophylactic therapy (70 vs. 123 mg/day), and the bleeding rate in patients taking propranolol was unusually high. In fact this study behaves as an outlier with respect to the other six showing almost equivalent effect of ligation and propranolol (Fig. 15.27). Therefore, there is no solid evidence to support the use of banding ligation as first-line prophylactic therapy, although it may be considered for patients with high-risk varices and contraindications to β-blockers (8).

Recommendations for clinical practice

The recommendations for prophylaxis of variceal bleeding are as follows (Fig. 15.28):

- Patients without varices should be screened endoscopically for the appearance of varices every 2 to

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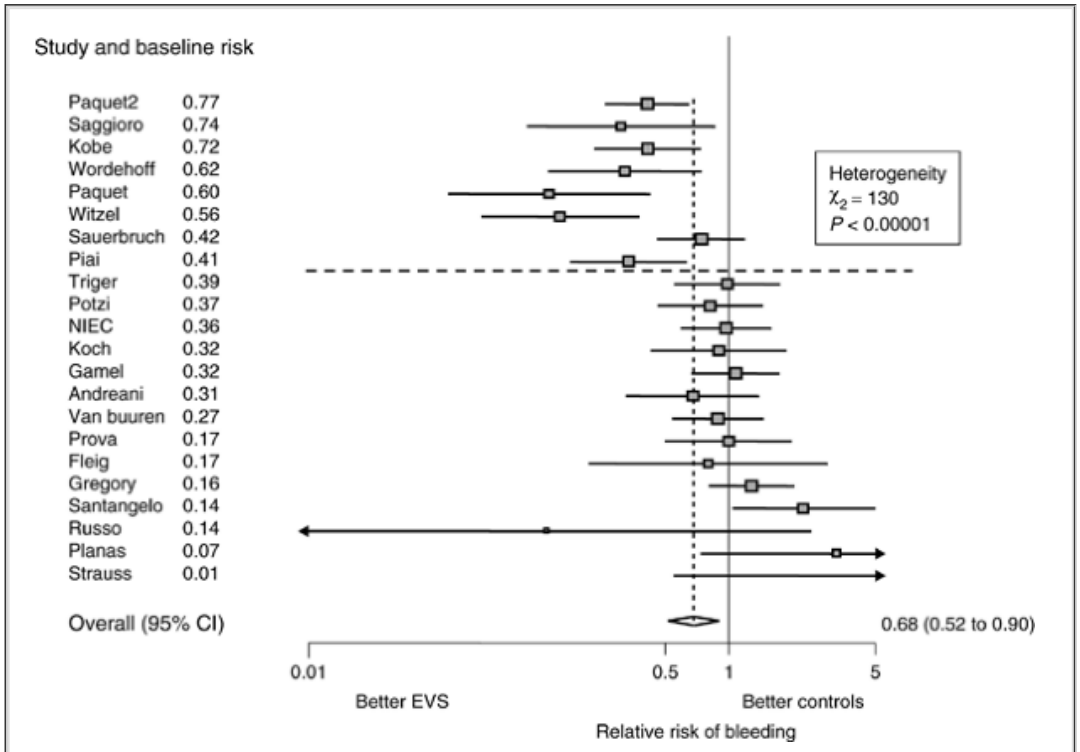
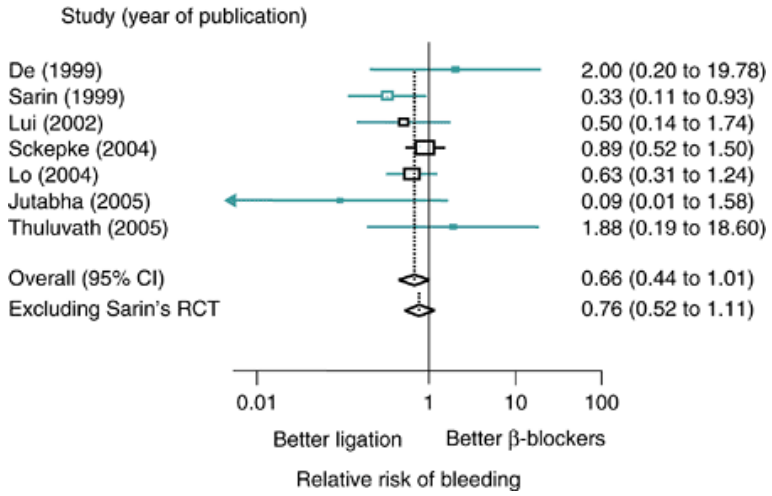
3 years. They should not receive treatment for prevention of the development of varices.

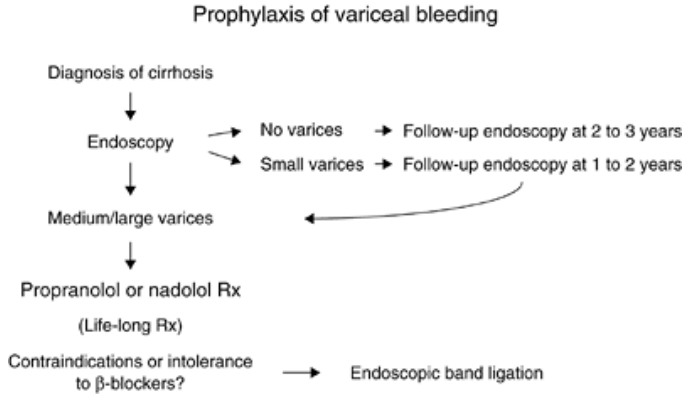


▪ **Figure 15.26** Meta-analysis of randomized clinical trials (RCTs) comparing endoscopic variceal sclerotherapy (EVS) with no treatment to prevent first bleeding in cirrhosis. RCTs are identified by the name of the first author (references in the text) and are sorted according to the baseline risk (the risk of bleeding among untreated controls). *Solid squares* indicate the relative risk for bleeding in the study groups for each RCT; the size of the solid squares varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the relative risks. The *vertical dashed line* represents the pooled relative risk of the whole set of RCTs. The *diamond* represents the 95% CI of the pooled relative risk. The *vertical solid line* represents the line of identity of effect of the two treatments (relative risk = 1). A marked and statistically significant difference was observed between RCTs, with a beneficial effect of

sclerotherapy when the baseline risk for bleeding was above 40%.



▪ **Figure 15.27** Meta-analysis of the randomized clinical trials (RCTs) comparing endoscopic band ligation of esophageal varices with β-blockers to prevent first bleeding in cirrhosis. Each RCT is identified by the name of the first author and year of publication (references in the text). *Squares* indicate the relative risk for bleeding with the two treatments for each RCT. The size of the square varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the relative risks. The *vertical dashed lines* represent the pooled relative risk of the whole set of RCTs or with exclusion of the RCT of Sarin (225). The *diamonds* represent 95% CIs of the pooled relative risk. The *vertical solid line* represents the line of identity of effect of the two treatments (relative risk = 1). The Sarin study appears to behave as an outlier, probably because of an insufficient dose of β-blocker (see text).



▪ **Figure 15.28** Recommended approach to the prophylaxis of variceal bleeding. All patients with cirrhosis should undergo diagnostic endoscopy. If medium to large varices are present, the patient should receive prophylactic

therapy with propranolol or nadolol unless these are contraindicated or the patient cannot tolerate them. In such cases endoscopic band ligation can be used. Patients with no varices or small varices should undergo follow-up endoscopy to assess the progression of the varices and determine the need for prophylactic treatment. Rx, treatment.

- Patients with small varices should be screened for enlargement of varices every 1 to 2 years. At present, there is no evidence to recommend treatment for the prevention of variceal bleeding in these patients because of their low risk of bleeding, but more studies are required to address this issue because approximately one third of the first episodes of variceal bleeding occur in patients with small varices and because drug therapy is the only applicable treatment in this situation.
- Patients with medium or large varices should be treated with a nonselective β-blocker. The dose should be titrated individually.
- Available evidence does not support the use of IMN alone or in combination with nonselective β-blockers.
- Endoscopic banding ligation appears to be an alternative to nonselective β-blockers. Patients with medium or large varices with contraindications or who are intolerant to β-blockers may be considered for banding ligation of varices. IMN alone does not appear to be a good treatment option for these patients.

Treatment of Acute Bleeding from Esophageal Varices

General management

Variceal bleeding is a medical emergency and its management should be undertaken in an intensive care setting by a team of experienced medical staff, including a clinical hepatologist, endoscopist, and surgeon. It is essential to have well-trained nurses. A specific plan of management should always be used, following a protocol adapted to the particular resources available. This is greatly facilitated by the establishment of gastrointestinal bleeding units. Early referral is warranted if there is a lack of either medical or surgical expertise in techniques to control bleeding (5).

Clinical and laboratory data to assess the severity of hemorrhage and liver disease should be included in the initial assessment because these have prognostic significance. The following should be evaluated: Hemoglobin/hematocrit, white cell count, platelet count, plasma urea and electrolytes, creatinine, prothrombin and partial thromboplastin times, baseline liver function tests, routine blood cultures, urine culture, ascitic fluid culture, chest radiograph, and electrocardiogram. Blood gases (in decompensated patients and in those with chest problems) should also be measured.

The initial therapy is aimed at correcting hypovolemic shock, preventing complications associated with gastrointestinal bleeding, and achieving hemostasis at the bleeding site. The two initial goals, which are independent of the cause of the hemorrhage, demand immediate management. Specific therapy to stop bleeding is usually given after the patient is subjected to the initial resuscitation and following diagnostic endoscopy, with the important exception of pharmacologic therapy, which can be started earlier in the course of the bleeding episode, on arrival to the hospital or even during transfer to hospital.

Replacement of blood volume

Variceal bleeding in cirrhosis is often massive; it is therefore essential to place at least one large-bore intravenous cannula to allow rapid blood transfusion if required. In addition, a central venous line should be inserted for monitoring the central venous pressure. The blood bank should be contacted on admission and four units of blood should be kept cross-matched and ready for transfusion at any time during the first 5 days (232).

Blood volume replacement should be initiated as soon as possible to avoid complications from hypovolemic shock and decreased perfusion of vital organs. It is important to emphasize that replacement of blood volume should be done conservatively and not considered solely in terms of blood transfusion. It is recommended to use plasma proteins or colloid expanders to replace the volume loss (at a sufficient rate to restore the systolic blood pressure to approximately 100 mm Hg, heart rate below 110 beats/minute, and the central venous pressure between 2 to 6 cm H_2O), while whole blood or packed red cells should be used conservatively to maintain the hemoglobin at about 8 g/dL (8). In this approach only a few units of blood may be needed. Frequent (hourly) measurements of blood pressure, heart rate, and central venous pressure during therapy are mandatory. The hematocrit/hemoglobin should be measured every 6 to 8 hours. Overtransfusion should be avoided, not only because of the risks inherent with blood transfusion but also because it may increase portal pressure, with a consequent risk of continued bleeding or rebleeding (233). Experimental studies in rats with portal hypertension have shown an increase in portal pressure beyond basal values when restoring the lost blood volume (233,234,235). Furthermore, hypovolemia and low blood pressure are powerful stimuli for the activation of vasoactive neurohumoral systems aimed at maintaining the arterial pressure within normal limits. This is achieved by a marked systemic vasoconstriction, which is especially accentuated in the splanchnic area, resulting in a reduction in portal–collateral blood flow with a concomitant fall in portal pressure. Therefore, variceal bleeding tends to stop spontaneously during hypovolemia (233) but may continue or start again with overtransfusion of any fluids. Indeed, studies in experimental models of portal hypertensive–related bleeding have shown that restoring 40% of the estimated blood loss using whole blood and polygeline (50:50) is associated with less blood loss and reduced mortality, as compared with strategies aimed at restoring all the blood volume loss. However, it is important to progressively correct anemia on the following days because persistent anemia is associated with worsening of the hyperkinetic syndrome and portal hypertension and may be a risk factor for rebleeding (236).

Prevention of complications

The main complications associated with gastrointestinal bleeding in patients with chronic liver disease are airway aspiration causing pneumonia, hepatic encephalopathy, infections by enteric organisms, hypoxemia, and renal/electrolyte imbalance, with an increase in the incidence of ascites and renal failure. These frequently lead to deterioration of liver function and may be the ultimate cause of death.

Aspiration of blood or gastric secretions is especially frequent in patients with impaired consciousness due to shock or hepatic encephalopathy. Although aspiration may occur at any time during the bleeding episode, the highest risks are during hematemesis, and during emergency endoscopy and placement of balloon tamponade tubes. Suspected or proved aspiration should be treated immediately with antibiotics; monitoring with pulse oximetry is useful. Its prevention is based on the following measures: (a) Close supervision by a well-trained nurse; (b) positioning the patient in a semireclined position, preferably the left lateral decubitus; (c) orotracheal intubation in comatose patients; and (d) aspiration of the gastric contents through an indwelling nasogastric

tube.

Nasogastric aspiration through an indwelling large bore tube is not universally used; however, it allows the enteral administration of drugs in comatose patients and is a useful method of monitoring the activity of bleeding. In addition, removal of blood from the upper gastrointestinal tract may help prevent and correct hepatic encephalopathy, which is a frequent complication of variceal hemorrhage. When present, encephalopathy is usually treated with the administration of lactulose or lactitol through nasogastric tube and cleansing enemas.

Support of vital organ function relies on the maintenance of adequate tissue perfusion and oxygenation. Aspiration of blood, prolonged shock, and multiple blood transfusions may markedly impair pulmonary gas exchange and result in significant hypoxemia and acute respiratory failure. Arterial hypoxemia due to hepatopulmonary syndrome is frequent in patients with cirrhosis who have advanced liver failure. This results from functional abnormalities such as pulmonary vasodilatation, impaired hypoxic vasoconstriction, ventilation/perfusion mismatching, and shunts. Therefore, it is essential to perform close hemodynamic monitoring, measurements of blood gases or pulse oximetry, and serial chest radiographs. Chest physiotherapy and provision of an adequate oxygen supply are integral parts of the therapy.

Renal function should be supported by adequate fluid and electrolyte replacement (saline infusions should be avoided) and should be monitored with strict attention to fluid balance. Close hemodynamic monitoring should prevent hypotension and allow rapid

correction of hypovolemic shock. It is crucial to avoid the administration of nephrotoxic drugs, especially aminoglycosides and nonsteroidal anti-inflammatory agents. Daily measurements of serum creatinine, urea, and electrolyte concentrations and the rate of urinary output are used as markers of renal perfusion. Urine output should be maintained at over 40 mL/hour; an output below 20 mL/hour indicates poor renal perfusion and impending renal failure. Massive or tense ascites should be treated with paracentesis, preferably with albumin replacement. This has been shown to improve the hemodynamic conditions and transiently decrease portal pressure and portal–collateral blood flow (237,238).

Patients with advanced cirrhosis frequently show wasting of muscle and adipose tissue. Malnutrition may contribute to an increased susceptibility to infections and impaired renal function. Suppression of oral intake during the acute bleeding episode significantly worsens the nutritional state; feeding should be resumed as soon as a 24-hour interval free of bleeding has been achieved. There are difficulties in providing a balanced nutrition in patients with cirrhosis. Enteral nutrition is always preferable because parenteral nutrition further complicates fluid balance and leads to an added risk of sepsis.

Alcoholic patients may exhibit withdrawal symptoms when admitted because of bleeding. These patients often require sedation. Close supervision of fluid and electrolyte balance is mandatory.

An area under investigation is whether the coagulation defects of cirrhosis, such as thrombopenia and factor VII deficiency, should be corrected in patients bleeding from esophageal varices. A double-blind RCT has recently shown that the administration of recombinant factor VIIa may significantly decrease the rate of failure to control variceal bleeding in the subgroup of patients with cirrhosis from Child-Pugh B and C classes (239). However the finding needs to be further confirmed before this treatment is recommended.

Prophylactic antibiotics

Up to 20% of patients with bleeding have a bacterial infection at the time of hospital

admission, and the risk of a nosocomial bacterial infection is nearly 50% in these patients compared to 5% to 7% of hospital-acquired infections in the general population (240,241). Furthermore, bacterial infections significantly increase the risk of failure to control bleeding and the hospital mortality rate for patients with cirrhosis and gastrointestinal bleeding (139,241). The most frequent bacterial infections seen in patients with cirrhosis are urinary tract infection (12% to 29%, mostly *Escherichia coli* or *Klebsiella*), spontaneous bacterial peritonitis (7% to 23%, gram-negative bacilli and aerobic gram-positive cocci), respiratory tract infection (6% to 10%), and primary bacteremia (4% to 11%). The risk of infection is higher for patients undergoing invasive procedures, such as endoscopy, which appears to be associated with bacteremia in 5% to 30% of cases (240).

A recent meta-analysis (242) including five RCTs (534 patients) has shown that antibiotic prophylaxis decreases the incidence of bacterial infections and increases the survival rate in patients admitted because of variceal bleeding. An updated meta-analysis of the eight RCTs now available (789 patients) confirms a significant beneficial effect of antibiotic prophylaxis in decreasing both mortality (RR 0.73, 95% CI 0.55 to 0.95) and the incidence of bacterial infections (RR 0.40, 95% CI 0.32 to 0.51) bacteremia, pneumonia, spontaneous bacterial peritonitis, and urinary tract infection (243).

The antibiotics assessed in these studies were fluoroquinolones, amoxicillin + clavulanic acid, ceftriaxone, imipenem + cilastin, and oral nonabsorbable antibiotics (e.g., gentamicin, vancomycin, nystatin).

Following these studies, a recent consensus conference stated that antibiotic prophylaxis should be an integral part of the therapy for acute gastrointestinal bleeding in cirrhosis and should be instituted from admission (8).

Pharmacologic treatment

The drugs assessed for acute bleeding are vasopressin and its analog terlipressin, and somatostatin and its analogs octreotide and vapreotide. Vasopressin and terlipressin have been combined with NTG in some trials.

Vasopressin

Vasopressin causes a marked splanchnic vasoconstriction with a pronounced decrease of portal blood flow and portal pressure. It also reduces portal–collateral blood flow and variceal pressure (182). However, it also causes marked systemic vasoconstriction, which may result in serious adverse events that prompt discontinuation of therapy in approximately 25% of the cases (5,182,184).

Severe arrhythmias, myocardial infarction, respiratory failure, cerebrovascular accidents, bowel necrosis, local tissue necrosis, and hyponatremia due to antidiuresis have been reported as side effects of vasopressin. Fatal complications have been rarely reported. Vasopressin has been abandoned because of these inconveniences and after the development of more effective and safer drugs.

Combination of NTG with vasopressin infusion significantly reduces the side effects, preventing one harmful event in every three treated patients, compared with

vasopressin alone (141). Moreover this combination also improves control of bleeding, although it does not significantly improve survival. The IV vasopressin infusion rate should be 0.4 U/minute. NTG should be simultaneously infused intravenously or, preferably, administered transdermically (20 mg/day) provided the systolic arterial pressure is over 100 mm Hg.

Terlipressin

Terlipressin is a long-acting vasopressin derivative with much less potential for severe side effects. Indeed, clinical studies have consistently shown less frequent and severe side effects with terlipressin than with vasopressin, even when vasopressin is combined with NTG (184). The increase in arterial pressure and the splanchnic vasoconstriction caused by terlipressin is associated with a significant improvement of renal function in patients with hepatorenal syndrome. Therefore, an additional advantage of this drug is that it may protect against the appearance of hepatorenal syndrome in patients with advanced cirrhosis and variceal bleeding (244,245,246,247).

Compared with placebo or nonactive treatment, terlipressin significantly improves the rate of control of bleeding and survival, with one death prevented in every six patients treated (248). This is the only treatment that has been shown to improve the prognosis of variceal bleeding in placebo-controlled RCTs and meta-analysis (249).

Terlipressin is used in bolus IV injections at doses of 2 mg every 4 to 6 hours for up to 48 hours. After achieving an initial control of bleeding (a 24-hour bleeding-free period), the dose can be halved and the treatment maintained for 5 days to prevent early rebleeding. The more frequent side effects are relatively mild: Abdominal cramps, diarrhea, bradycardia, and hypertension. Severe side effects (e.g., arrhythmias, angina, cerebrovascular accident, and limb ischemia) requiring discontinuation of the drug are in the order of 2% to 4% (232,248).

Terlipressin has been compared with vasopressin in five trials. Vasopressin has been combined with transdermal or sublingual NTG in two studies (141). Overall, these studies did not show significant differences between the two drugs in the control of bleeding, rebleeding, or mortality, but side effects were significantly less frequent and less severe with terlipressin. Terlipressin is as effective as somatostatin infusion and endoscopic therapy (see subsequent text). The overall efficacy of terlipressin in controlling acute variceal bleeding is 75% to 80% across trials (248).

Somatostatin

Several RCTs showed that somatostatin compared with placebo or nonactive treatment significantly improves the rate of control of bleeding. Although the results of these studies were heterogeneous, their meta-analysis showed that this heterogeneity was mainly due to one study with an unusually high rate of spontaneous bleeding cessation (83%, the highest ever reported) in the placebo-treated group (141). However, despite the beneficial effect on control of bleeding, somatostatin does not affect mortality.

Compared with vasopressin, somatostatin is equivalent for mortality and control of bleeding but is associated with less frequent and less severe side effects than vasopressin (141).

Somatostatin has been compared with terlipressin in three studies, including a total of 302 patients. The two larger studies were double-blind placebo-controlled RCTs. Overall, no differences were found for failure to control bleeding, rebleeding, and mortality. Total side effects were 21% with somatostatin versus 29% with terlipressin (NS), and major side effects requiring withdrawal of treatment or specific therapy were 4% in both treatment groups (141).

Contrary to what has been shown for vasopressin, addition of NTG to somatostatin does not improve therapeutic efficacy and induces more adverse effects.

Somatostatin is used as a continuous IV infusion of 250 μg/hour following an initial bolus of 250 μg. A recent study suggested that in high-risk patients (with active bleeding at the time of diagnostic endoscopy) it is wise to increase the infusion dose to 500 μg/hour and to provide repeat 250 μg boluses during the initial hours of therapy (187). Treatment may be maintained for up to 5 days. Side effects of somatostatin are usually mild, most frequently bradycardia, hyperglycemia, diarrhea, and abdominal cramps.

Octreotide

Octreotide is a synthetic somatostatin analog with longer half-life. Like somatostatin, bolus injections of octreotide cause a transient increase in mean arterial pressure and systemic vascular resistance, suggesting a systemic effect (250). However, at the doses used empirically to treat variceal hemorrhage, or higher doses, octreotide infusions have not been found to cause a sustained decrease in portal pressure or collateral blood flow. A rapid desensitization of the hemodynamic effects of octreotide has been reported after its intravenous administration (250). Therefore, the hemodynamic effects of octreotide, as well as its best dose and route of administration, remain unclear. In many occasions, its use has been empirical, assuming that it should be similar to somatostatin, which is probably not exact.

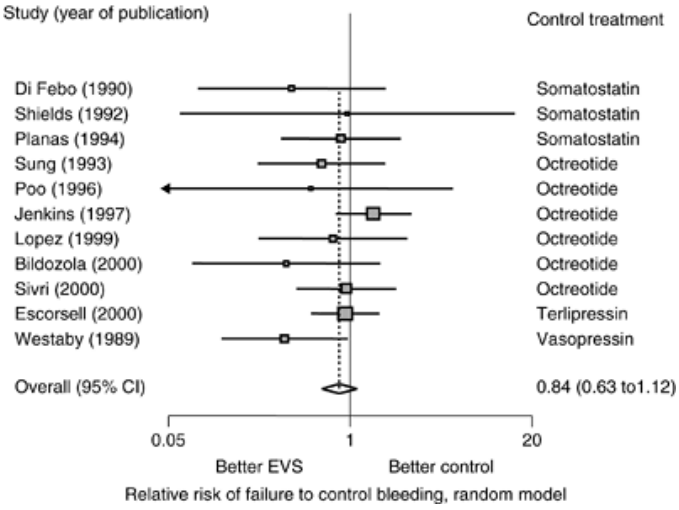
The efficacy of octreotide as a single therapy for variceal bleeding is controversial. Among four RCTs comparing octreotide with placebo, no benefit from

octreotide was found in the only one using octreotide or placebo as initial treatment, whereas in the three using sclerotherapy or ligation before or at the same time of octreotide administration, a significant benefit was found in two studies and nearly significant in the third (141). These results suggest that octreotide may improve the results of endoscopic therapy but has no or little effect if used alone. No effects of octreotide on rebleeding or death were found (141). When compared with other vasoactive drugs, octreotide was better than vasopressin for control of bleeding in two RCTs and similar to terlipressin in other two, again suggesting a clinical value for the use of octreotide. However, the studies were not sized to test for equivalence. Side effects were less frequent and severe with octreotide than with either vasopressin or terlipressin, but the difference was significant only for vasopressin (141).

Octreotide has been used as continuous IV infusions, at empirical dosages of 25 to 50 µg/hour, in some instances after initial IV boluses of 50 µg. Treatment duration was from 1 to 5 days.

Vapreotide

Vapreotide is another somatostatin analog that was shown to improve control of variceal bleeding and transfusion requirements in a double-blind RCT (251). Further studies are required before clinical use of vapreotide can be recommended.



▪ **Figure 15.29** Meta-analysis of the randomized clinical trials (RCTs) comparing emergency endoscopic variceal sclerotherapy (EVS) with vasoactive drugs to

control acute variceal bleeding in cirrhosis. Each RCT is identified by the name of the first author and year of publication; control treatments are listed on the right-hand side of the grid. *Solid squares* indicate the relative risk for failure to control bleeding with the two treatments for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the pooled relative risks. The *vertical dashed line* represents the pooled relative risk of the whole set of RCTs. The *diamond* represents the 95% CI of the pooled relative risk. The *vertical solid line* represents the line of identity of effect of the two treatments (relative risk = 1). The meta-analysis shows that vasoactive drugs are equivalent to emergency sclerotherapy for the control of acute variceal bleeding.

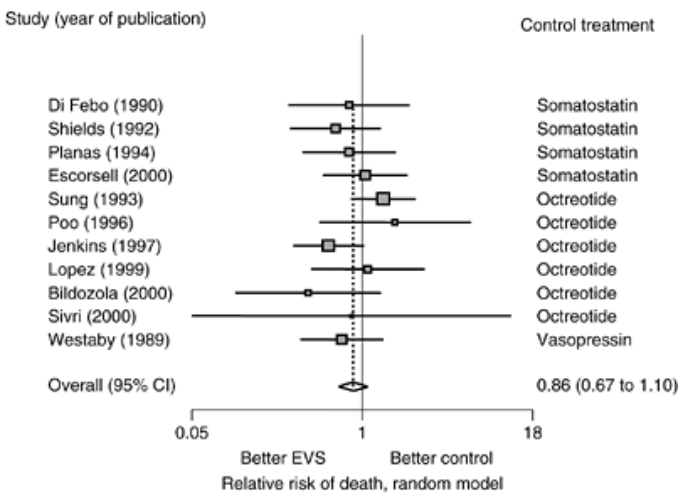
Endoscopic treatment

Emergency endoscopic injection sclerotherapy of esophageal varices is widely used as first-choice treatment because it stops bleeding in about 80% to 90% of patients. Moreover, it allows the start of specific treatment for the prevention of long-term rebleeding at the time of diagnostic endoscopy. However, it requires a skilled endoscopist and is associated with serious complications in 10% to 20% of patients, with an overall mortality of 2% (141).

Emergency sclerotherapy significantly improves control of bleeding and survival when compared with sham therapy (249). However when compared with vasoactive drugs, it is superior only to vasopressin for controlling bleeding, but is similar to terlipressin, somatostatin, or octreotide either for control of bleeding/early rebleeding or for mortality (Figs. 15.29 and 15.30) (252). The equivalence between sclerotherapy and pharmacologic therapy is confirmed also by pooling RCTs irrespective of the vasoactive control therapy (252).

Emergency banding ligation of esophageal varices is probably equivalent to emergency sclerotherapy (218). There is only one RCT specifically designed to compare the two techniques for controlling acute variceal bleeding (253), while eight RCTs for long-term prevention of recurrent variceal bleeding allow for the comparison of the two techniques used

in the emergency situation in the subgroups of patients included with active variceal bleeding (218). The meta-analysis of all these studies, including 288 patients overall, failed to show any significant difference between the two endoscopic techniques either in control of bleeding or in mortality (218). However, it has been reported that emergency banding may be more difficult than sclerotherapy because the field of vision is reduced by approximately 30% with the banding device attached, and blood in the esophagus may further reduce vision and hamper the effective placement of the rubber bands. Sclerotherapy does not have this limitation but carries a greater potential for complications than does banding ligation using multiband devices. The choice of one or another procedure may depend on the expertise with each of these techniques.



▪ **Figure 15.30** Meta-analysis of the randomized clinical trials (RCTs) comparing emergency endoscopic variceal sclerotherapy (EVS) with vasoactive drugs for mortality in patients with acute variceal bleeding in cirrhosis. Each RCT is identified by the name of the first author and year of publication; control treatments are listed on the right-hand side of the grid. *Solid squares* indicate the relative risk for death with the two treatments for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the relative risks. The *vertical dashed line* represents the pooled relative risk of the whole set of RCTs. The *diamond* represents the 95% CI of the pooled relative risk. The *vertical solid line* represents the line of identity of effect of the two treatments (relative risk = 1). The meta-analysis shows that vasoactive drugs are equivalent to emergency sclerotherapy for mortality in patients with acute variceal bleeding. IMN, isosorbide-5-mononitrate.

Endoscopic obturation of esophageal varices by means of the injection of tissue adhesives does not offer advantages compared with sclerotherapy or band ligation (254), and in one study it was associated with a higher incidence of complications than ligation (218). However, the combination of obturation and sclerotherapy may be superior to sclerotherapy alone, suggesting that obturation may be an option in patients in whom bleeding is not controlled by sclerotherapy (218). This treatment has found its best use in patients bleeding from gastric varices (see subsequent text).

Combined endoscopic and pharmacologic therapy

The combination of endoscopic therapy (either sclerotherapy or banding) with pharmacologic therapy significantly improves initial control of bleeding and reduces the 5-day failure rate in terms of control of bleeding or rebleeding (255,256) when compared to endoscopic therapy alone. However, the combination therapy does not improve 5- or 42-day mortality (255,256), while it significantly increases adverse effects (252).

Notably, only two of the RCTs assessing the efficacy of combined endoscopic and pharmacologic therapy used banding ligation, which is nowadays the standard of endoscopic therapy for esophageal varices for clinical practice. Moreover, the advantage of a better initial and 5-day control of bleeding has not translated into survival improvement. Therefore, the advantages of the combination of pharmacologic and endoscopic therapy should be further explored in large RCTs.

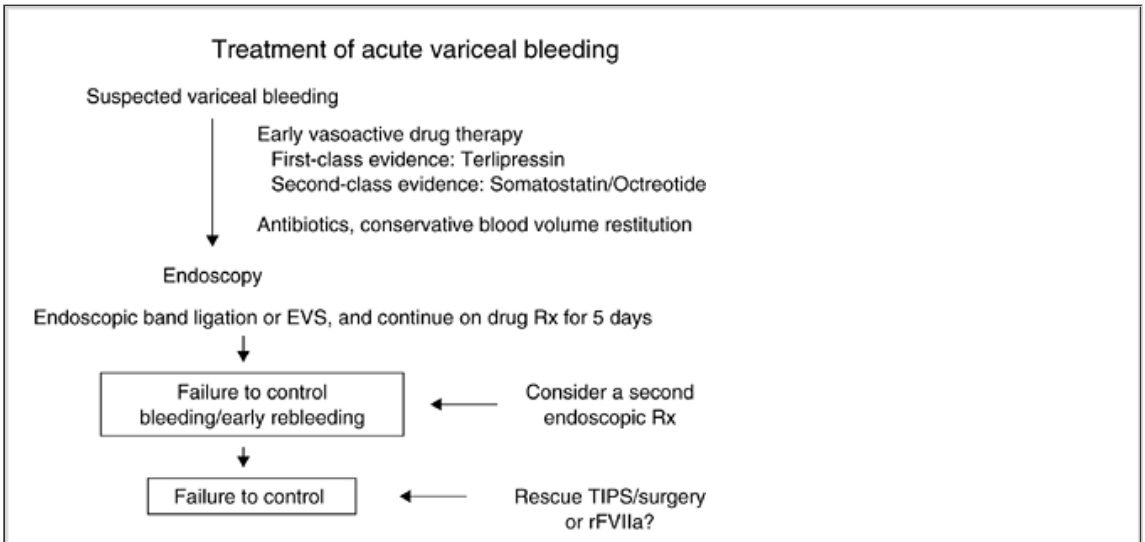
Balloon tamponade

Balloon tamponade stops bleeding by direct compression of the bleeding site at the varices. Control of bleeding is successful in as much as 80% to 90%, but up to 50% of patients will rebleed when the balloon(s) is(are)

deflated. Its efficacy has been confirmed by several RCTs showing that it is more effective than vasopressin and as effective as terlipressin (Glypressin), somatostatin, octreotide, and sclerotherapy (249). Complications are frequent and may be lethal in 6% to 20% of cases. These include aspiration pneumonia, large esophageal ulcers, esophageal rupture, and airways obstruction. Because of the frequency and severity of complications and the high rate of rebleeding, balloon tamponade should be used only by skilled and experienced staff in intensive care units only for temporarily controlling variceal bleeding that is uncontrollable by other treatments, while waiting for definitive therapy.

Transjugular intrahepatic portosystemic shunt

TIPS, when used for acute variceal hemorrhage, stops bleeding in most patients. Some centers use coil embolization of the vessels feeding the varices. However, rebleeding is far more common than after elective TIPS (257). Selection of patients with uncontrolled bleeding, many of them with advanced liver failure, probably accounts for the high 6-week mortality, ranging from 27% to 55% (257). An RCT has recently shown that the best candidates for emergency TIPS for variceal bleeding are those with HVPG ≥20 mm Hg in whom TIPS reduced the 6-week mortality from 38% to 17%, while the corresponding figure was 5% in patients with HVPG less than 20 mm Hg not treated by TIPS (258). Although the applicability of the results of this trial to clinical practice is questionable because it requires HVPG measurement early during the episode of variceal bleeding, it indicates that high-risk patients will benefit from TIPS if they may be treated soon, before clinical conditions deteriorate. Whether other clinical risk indicators may be used in clinical practice instead of HVPG measurement should be assessed in future RCTs. Until such studies are available, given the very high proportion of patients in whom variceal bleeding is controlled by medical and/or endoscopic therapy, TIPS is confined to the rare patients (approximately 10% of cases) with uncontrollable variceal bleeding.



▪ **Figure 15.31** Recommended approach to the treatment of acute variceal bleeding. As soon as possible, patients in whom variceal bleeding is suspected should receive vasoactive drug therapy, even before they arrive at the hospital. At

endoscopy, band ligation or endoscopic variceal sclerotherapy (EVS) should be performed, and drug therapy should be maintained for 5 days to prevent early rebleeding. If bleeding is not arrested or recurs, a second attempt at endoscopic therapy should be considered. If bleeding continues, a rescue transjugular intrahepatic portosystemic shunt (TIPS) or surgical decompression may be indicated. Ancillary therapy with recombinant activated factor VII (rF-VIIa) may be considered in patients with liver failure too advanced to be treated with TIPS. Rx, treatment.

Surgery

Following the introduction of TIPS in clinical practice, shunt surgery and devascularization procedures have almost been abandoned in many centers. However, it is possible that the rare Child-Pugh A or B patient with uncontrollable variceal bleeding may be better managed surgically (using interposition Gore-Tex mesocaval graft shunt) than with TIPS, provided the center still has well-trained surgeons for shunt surgery, which is not frequent at present (see also Chapter 17).

Recommendations for clinical practice

The recommendations for treatment of variceal rebleeding are as follows (Fig. 15.31):

- Blood volume restitution should be done cautiously to maintain hemodynamic stability and hemoglobin at approximately 8 g/dL.

- Antibiotic prophylaxis against gram-negative bacilli and gram-positive cocci should be instituted from admission.
- Pharmacologic treatment should be started immediately when variceal bleeding is suspected, even before endoscopic confirmation of diagnosis.
- Terlipressin (in countries where it is available) is the first choice of treatment because it may reduce mortality. Somatostatin may be considered as an alternative to terlipressin. An increased dose and the administration of extra bolus injections should be considered in patients who actively bleed during endoscopy.
- Octreotide improves the efficacy of emergency endoscopic therapy in controlling variceal bleeding. At present, there is no evidence supporting its use as single therapy.
- If vasopressin is used, it should be combined with nitroglycerin.
- Endoscopic therapy (preferably by banding ligation) is recommended as soon as an experienced endoscopist is available. Pharmacologic therapy should be maintained even after endoscopic therapy for 2 to 5 days.
- TIPS should be used in case of failures of medical and endoscopic therapy.
- Shunt surgery using interposition mesocaval graft shunts or traditional portacaval shunts may be an alternative to TIPS in Child-Pugh A patients (preferably in nontransplantation candidates).

Prevention of Recurrent Bleeding from Esophageal Varices

Patients surviving a first episode of variceal bleeding have a high risk of recurrent bleeding of over 60% within 1 year of the first bleeding. Because of this, all patients surviving a variceal bleeding should receive active treatment for the prevention of rebleeding (8).

Pharmacologic treatment

Pharmacologic treatment for the prevention of rebleeding is based on the use of nonselective β-blockers, which were introduced in the 1980s (179). Recently, their combination with IMN has been proposed (63,190).

Nonselective β-blockers

The hemodynamic basis for the use of nonselective β-blockers in the prevention of rebleeding, as well as their contraindications and side effects, is the same for the prevention of first bleeding and have been discussed earlier in this chapter.

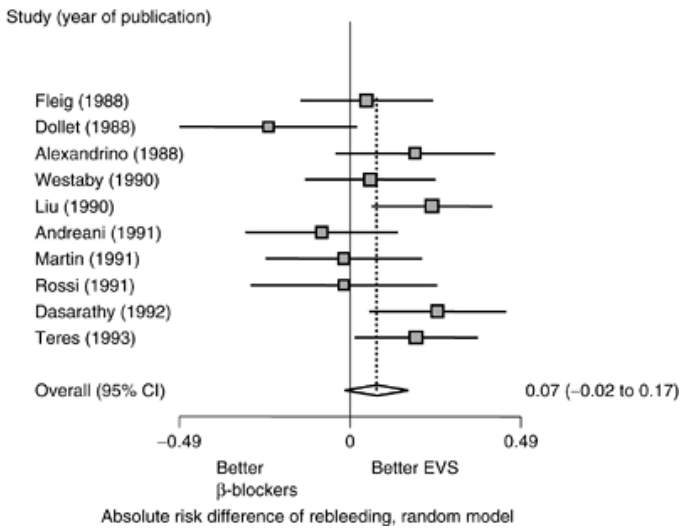
The efficacy of nonselective β-blockers in the prevention of variceal rebleeding has been proved by many RCTs, and these drugs are now widely accepted as the first-line pharmacologic therapy in this setting (8). Twelve RCTs including a total of 809 patients compared nonselective β-blockers with placebo or nonactive treatments. Several meta-analyses of these studies consistently found a marked benefit from β-blockers. The rebleeding rate is reduced from 63% in controls to 42% in treated patients, with an absolute risk reduction (ARD) of -21% (95% CI -30% to -13%) (141). This means that the number of patients needed to treat (NNT) to prevent one rebleeding episode is five. Mortality was significantly reduced, from 27% to 20% (ARD = -7%; 95% CI -12% to -2%; NNT = 14) (141,259). Mortality from bleeding was also significantly reduced by β-blockers (259).

β-Blockers were compared with endoscopic variceal sclerotherapy in ten RCTs including 862 patients. No significant differences were found between the two treatments either for rebleeding (ARD = 7%; 95% CI -2% to 17%) (Fig. 15.32) or for mortality (ARD = 2%; 95% CI -5% to 8%) (Fig. 15.33). Side effects were significantly less frequent and less severe with β-blockers (ARD = -22%; 95% CI -38% to -6%). The number of patients needed to be treated with β-blockers to prevent a harmful event (NNH), compared with sclerotherapy, is four (141).

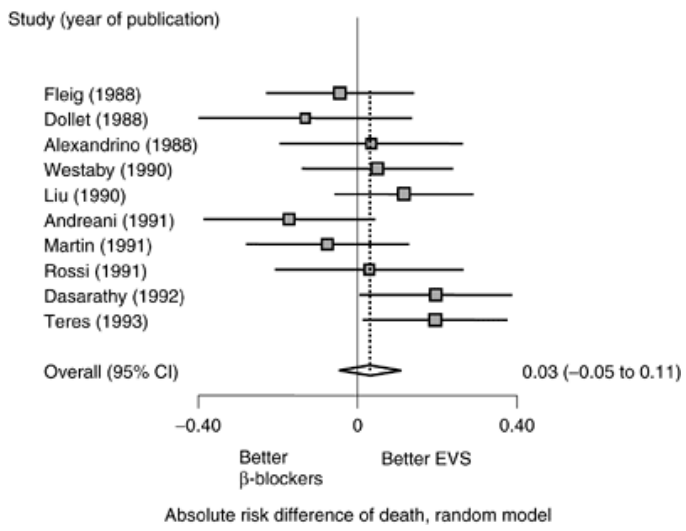
Combined pharmacologic treatment

Nonselective β-blockers plus organic nitrates—the combined administration of propranolol or nadolol plus IMN was introduced after demonstrating that IMN enhanced the portal pressure–reducing effect of nonselective β-blockers (190). There is insufficient information on whether this translates into a clinical advantage because there are only two studies of IMN combined with propranolol (260) or nadolol (261) versus the corresponding β-blocker alone in the prevention of rebleeding. One of the studies showed significant benefit, but not the other (which is still available only in abstract form) (261). However, the combination of IMN with propranolol or nadolol has been found to be superior to either endoscopic sclerotherapy in one study (262) or band ligation in three studies (263,264,265). When all the four studies are pooled (Fig. 15.34), a nonsignificant trend toward a bleeding risk reduction with the combination pharmacologic therapy is found. The results of the four studies are heterogeneous because one trial (265) found an opposite result (benefit with ligation). It is, however, notable that this trial included a significantly higher proportion of patients with large varices in the drug arm than in the endoscopic arm and that the dose of nitrate used was much lower than that in the other studies. These factors may, at least in part explain the heterogeneity. In fact, by removing this study from the meta-analysis, heterogeneity disappears and a significant bleeding risk reduction with drug therapy is

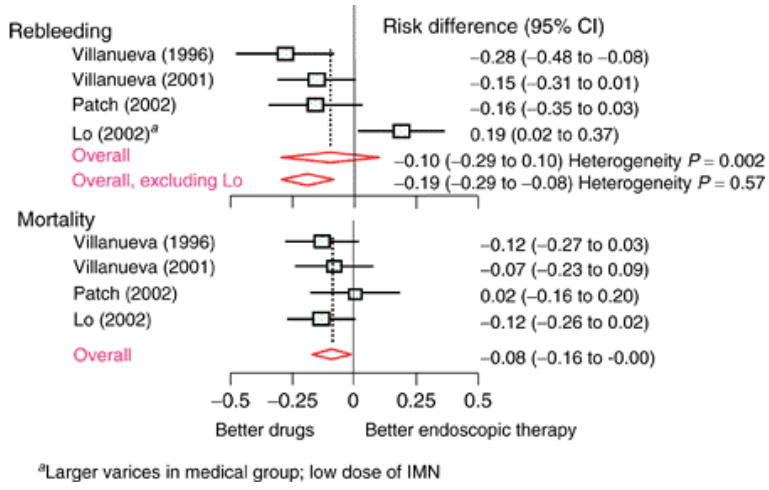
found (ARD = -0.19; 95% CI -0.29 to -0.08), with one bleeding episode prevented out of every five patients treated with the pharmacologic combination. Mortality was nonsignificantly reduced with the pharmacologic combination in three studies (262,264,265) and was the same in one (263). Altogether, mortality was almost significantly less with the combination of β-blocker plus IMN (ARD = -0.08; 95% CI -0.16 to 0.00) (Fig. 15.34). Compared with TIPS, the combination of IMN and propranolol is less effective for the prevention of rebleeding but is associated with significantly less encephalopathy, similar mortality, and much lower cost (266).



▪ **Figure 15.32** Meta-analysis of the randomized clinical trials (RCTs) comparing nonselective β-blockers with endoscopic variceal sclerotherapy (EVS) to prevent recurrent variceal bleeding in patients with cirrhosis. Each RCT is identified by the name of the first author and year of publication. *Solid squares* indicate the absolute difference in risk for recurrent bleeding with the two treatments for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the differences in risk. The *vertical dashed line* represents the pooled absolute difference in risk of the whole set of RCTs. The *diamond* represents the 95% CI of the pooled absolute difference in risk. The *vertical solid line* represents the line of identity of the effect of the two treatments (absolute difference in risk = 0). The meta-analysis shows that β-blockers and sclerotherapy are equivalent for the prevention of recurrent bleeding in cirrhosis.



▪ **Figure 15.33** Meta-analysis of the randomized clinical trials (RCTs) comparing nonselective β-blockers with endoscopic variceal sclerotherapy (EVS) for reducing mortality in patients with cirrhosis who survive an episode of variceal bleeding. Each RCT is identified by the name of the first author and year of publication. *Solid squares* indicate the absolute difference in risk for death with the two treatments for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the differences in risk. The *vertical dashed line* represents the pooled absolute difference in risk of the whole set of RCTs. The *diamond* represents the 95% CI of the pooled absolute difference in risk. The *vertical solid line* represents the line of identity of effect of the two treatments (absolute risk difference = 0). The meta-analysis shows that β-blockers and sclerotherapy are equivalent for mortality after the first episode of variceal bleeding in cirrhosis.



▪ **Figure 15.34** Meta-analysis of the randomized clinical trials (RCTs) comparing β-blockers + isosorbide-5-mononitrate (IMN) with endoscopic band ligation of esophageal varices to prevent recurrent bleeding from esophageal varices in cirrhosis. Each RCT is identified by the name of the first author and year of

publication (references in the text). *Squares* indicate the relative risk for bleeding with the two treatments for each RCT. The size of the *square* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the relative risks. The *vertical dashed lines* represent the pooled relative risk of the whole set of RCTs or with exclusion of the RCT of Lo (265). The *diamonds* represent 95% CIs of the pooled relative risk. The *vertical solid line* represents the line of identity of effect of the two treatments (relative risk = 1). The study of Lo appears to behave as an outlier, probably because of a different distribution of patients with large varices between the two treatment groups and insufficient dose of IMN (see text). *Upper panel*, absolute risk difference for rebleeding; *lower panel*, absolute risk difference for mortality.

Treatment dosage and schedules for propranolol, nadolol, and IMN are the same as those for the prevention of first bleeding.

Monitoring pharmacologic treatment response

Eight studies, either RCTs or prospective consecutive series (143,144,216,262,263,264,267,268), have shown that the pharmacologic (or spontaneous) reduction of HVPG to less than 12 mm Hg or by as much as or more than 20% of the baseline value virtually abolishes the risk of rebleeding. Seven of these studies showed a reduction in bleeding risk in patients achieving at least one of the two hemodynamic targets, and the reduction was significant in six. In only one study was no benefit found from HVPG reduction. When the results of these eight studies are combined in a meta-analysis, the odds ratio for rebleeding in patients achieving one of these hemodynamic targets compared to those not reaching any of them is 0.20 (95% CI 0.08 to 0.47). Therefore, the patients reaching one (or both) of these targets are considered hemodynamic responders to pharmacologic therapy. However, because it is still unclear whether patients with an insufficient hemodynamic response to pharmacologic therapy would benefit from alternative treatments, hemodynamic monitoring of pharmacologic therapy is presently recommended only in the setting of clinical research (8).

Endoscopic treatment

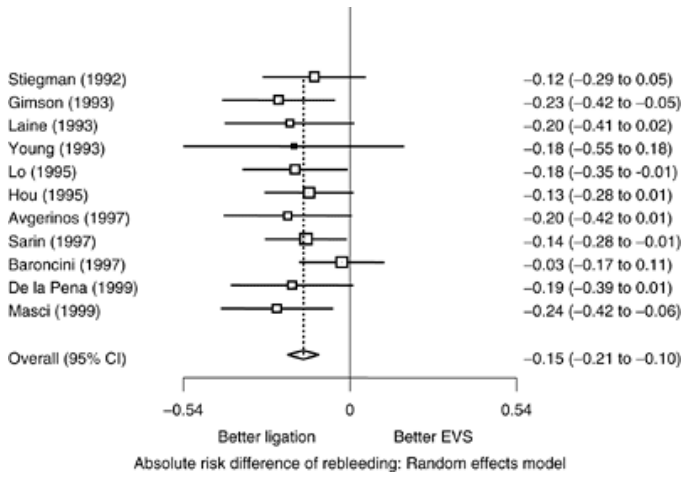
Endoscopic injection sclerotherapy of esophageal varices significantly reduces both the rebleeding and death risk (218), preventing one bleeding episode out of every seven treated patients and one death out of every ten. It takes four to six endoscopic sessions to eradicate varices, but recurrence of varices occurs in nearly 40% of patients within 1 year of eradication. This requires further endoscopic sessions to maintain eradication. It has been estimated that endoscopic sclerotherapy for the prevention of rebleeding may require one endoscopy per month per patient for the first 2 years of treatment. The most serious side effects of therapy are dysphagia, esophageal stenosis, and bleeding from esophageal ulcers, which may account for as much as 14% of all the rebleeding episodes. As commented in the preceding text, sclerotherapy has no advantage over drug therapy and causes more frequent and severe side effects.

Endoscopic banding ligation has been proved superior to sclerotherapy in 20 RCTs (1,634 patients), 11 published as full reports and 9 in abstract form (269) (Fig. 15.35) (270). The meta-analysis of the 11 fully published RCTs shows that the absolute bleeding risk difference is -15% (95% CI -21% to -10%). Complications are significantly less frequent and severe with banding ligation (218). Therefore, banding ligation should be preferred to sclerotherapy (8). Surprisingly, despite decreasing

rebleeding rates, endoscopic ligation does not significantly improve survival compared with sclerotherapy (pooled absolute death risk difference for the 11 fully published RCTs is -2%; 95% CI -7% to +3%), and there is evidence that it is associated with higher recurrence of varices (218).

Combined endoscopic treatment

Banding ligation combined with sclerotherapy—sclerotherapy has been added (either simultaneously or after the reduction of variceal size to small) to endoscopic band ligation and compared to band ligation alone in eight RCTs, yielding contrasting results (218). The meta-analysis of these studies does not show any benefit either for rebleeding (ARD = -0.06; 95% CI -0.17 to 0.05) or for mortality, and importantly, it shows a trend toward an increasing complication rate with combination endoscopic therapy.



▪ **Figure 15.35** Meta-analysis of the randomized clinical trials (RCTs) published as full reports and comparing endoscopic band ligation of esophageal varices with endoscopic variceal sclerotherapy (EVS) for the prevention of recurrent variceal bleeding in patients with cirrhosis. Each RCT is identified by the name of the first author and year of publication (references in the text). *Squares* indicate the absolute difference in risk for recurrent bleeding, with two treatments for each RCT. The size of the *square* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CIs) of the relative risks. The *vertical dashed line* represents the pooled absolute difference in risk of the whole set of RCTs. The *diamonds* represent 95% CIs of the pooled absolute difference in risk. The *vertical solid line* represents the line of identity of effect of the two treatments (absolute difference in risk = 0). The meta-analysis shows that endoscopic banding ligation of varices significantly reduces the risk for recurrent variceal bleeding.

Combined endoscopic and pharmacologic treatment

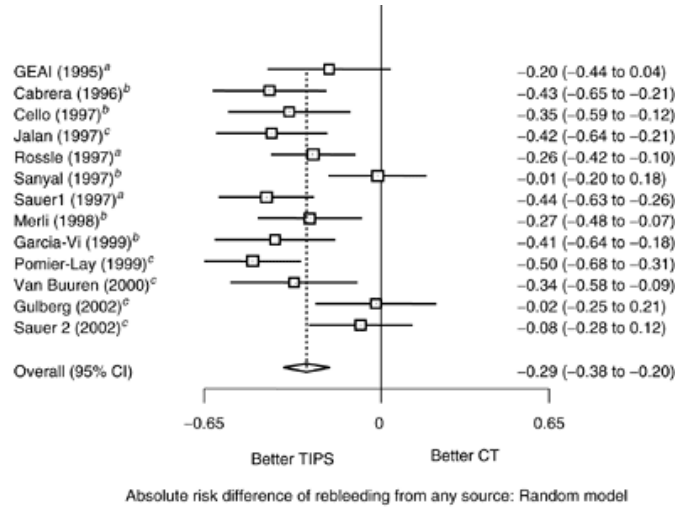
The combination of injection sclerotherapy and β-blockers has been compared with either sclerotherapy or β-blockers alone. The meta-analysis of the ten RCTs comparing combination therapy with sclerotherapy alone showed a significant reduction of the rebleeding risk (pooled odds ratio 0.66, 95% CI 0.46 to 0.93) with combination therapy, but no differences for mortality (pooled odds ratio 0.83, 95% CI 0.52 to 1.33) (249). Also, when compared with β-blockers alone, combination therapy significantly reduced the rebleeding risk without advantage in survival (141).

Two RCTs have shown that the combination of banding ligation plus β-blockers is superior to banding ligation alone in terms of recurrence of varices and recurrence of bleeding (271,272). Therefore, the combination of endoscopic therapy with a nonselective β-blocker is particularly recommended in patients who bleed under either treatment alone. In these patients, the substitution of the failing treatment with the other should not be preferred to their combination.

Transjugular intrahepatic portosystemic shunt

The efficacy of TIPS in the prevention of recurrent bleeding from esophageal varices is not much

different from that of shunt surgery, especially since the introduction of polytetrafluoroethylene (PTFE)-covered stents. It dramatically diminishes the risk of rebleeding (Fig. 15.36), but does not improve mortality and significantly increases the incidence of portosystemic encephalopathy (Fig. 15.37). TIPS has been compared with sclerotherapy in nine RCTs and with banding ligation in four RCTs (273). The 13 trials almost consistently showed that TIPS is superior to either endoscopic therapy for the prevention of rebleeding. When the results of these studies are pooled, the absolute rebleeding risk difference is -29% (95% CI -38% to -20%), indicating that one rebleeding would be prevented in every three patients treated by TIPS instead of endoscopic therapy. Not surprisingly, this impressive efficacy in preventing recurrent bleeding is accompanied by a marked increase in the risk of encephalopathy, with one extra episode of encephalopathy out of every seven patients treated by TIPS (ARD = 14%; 95% CI, 7% to 20%). Overall mortality and mortality from bleeding are similar with TIPS and endoscopic therapy.

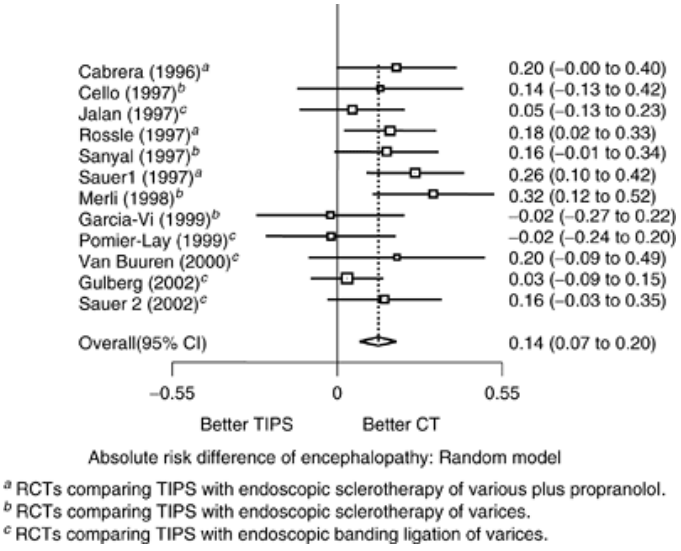


▪ **Figure 15.36** Meta-analysis of the randomized clinical trials (RCTs) of transjugular intrahepatic portosystemic shunt (TIPS) compared with endoscopic control therapy (CT) for the prevention of recurrent variceal bleeding in patients with cirrhosis, published as full report. Each RCT is identified by the name of the first author and year of publication. *Solid squares* indicate the absolute risk difference of recurrent bleeding with the two treatments for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CI) of risk differences. The *vertical dashed line* represents the pooled absolute risk difference of the whole set of RCTs. The *diamond* represents the 95% CIs of the pooled

absolute risk difference. The *vertical solid line* represents the line of identity of effect of the two treatments (absolute risk difference = 0). The meta-analysis shows that after a variceal bleeding episode TIPS significantly reduces the risk of recurrent bleeding compared with endoscopic therapy.

TIPS has been compared with surgical shunts in two RCTs (274,275). In the first it was compared with 8-mm portacaval H-graft shunt (274). A significantly lower rebleeding rate was found with the surgical shunt. Significantly more patients required liver transplantation in the TIPS group than in the surgical shunt group. There was no difference in mortality. The composite endpoint of "failures," which included rebleeding, shunt thrombosis, deaths, and need for transplant, was significantly higher for TIPS.

In the second trial, TIPS was compared with the DSRS in Child-Pugh class A and B patients (275). The rebleeding rate was 5.5% in the DSRS group and 9% in the TIPS group (P = 0.27). The reintervention rate was significantly higher (P = 0.001) in the TIPS group (82%) than in the DSRS group (11%). At least one episode of encephalopathy was observed in 50% of patients in each group, and cumulative two 2-year survival was over 80% and 5-year survival was over 60% in both groups. Seven patients in each group underwent transplantation.



▪ **Figure 15.37** Meta-analysis of the randomized clinical trials (RCTs) of transjugular intrahepatic portosystemic shunt (TIPS) compared with endoscopic control therapy (CT) for the prevention of recurrent variceal bleeding in patients with cirrhosis. The risk of developing portosystemic encephalopathy is evaluated. Each RCT is identified by the name of the first author and year of publication. *Solid squares* indicate the absolute risk difference of encephalopathy for each RCT; the size of the *solid squares* varies according to the weight of each study in the meta-analysis. *Horizontal bars* denote the 95% confidence intervals (CI) of risk differences. The *vertical dashed line* represents the pooled absolute risk difference of the whole set of RCTs. The *diamond* represents the 95% CIs of the pooled absolute risk difference. The *vertical solid line* represents the line of identity of effect of the two treatments (absolute risk difference = 0). The meta-analysis shows that TIPS significantly increases the risk of encephalopathy compared with

endoscopic therapy.

These two RCTs (274,275) indicate that although variceal rebleeding may be lower with the surgical shunt, in low-risk patients (Child-Pugh class A and B) surgical shunt or TIPS do not significantly differ in the rate or severity of encephalopathy, progression of liver disease, and long-term survival. TIPS needs significantly more surveillance and intervention to maintain patency and achieve these good long-term results.

A major drawback of TIPS is the high rate of occlusion or dysfunction at 2 years, in the order of 60% to 80%. However, one recent multicenter randomized trial (201) and two studies including consecutive patients with Budd-Chiari syndrome (276) or cirrhosis (277) reported much lower obstruction and reintervention rates with PTFE-covered stents and lower rates of recurrent bleeding or ascites, without increased incidence of encephalopathy (201). These results suggest that the small disadvantage of TIPS versus surgical shunt would be overcome by the use of PTFE-covered stents and that uncovered stents will be probably abandoned in the next future.

Recommendations for clinical practice

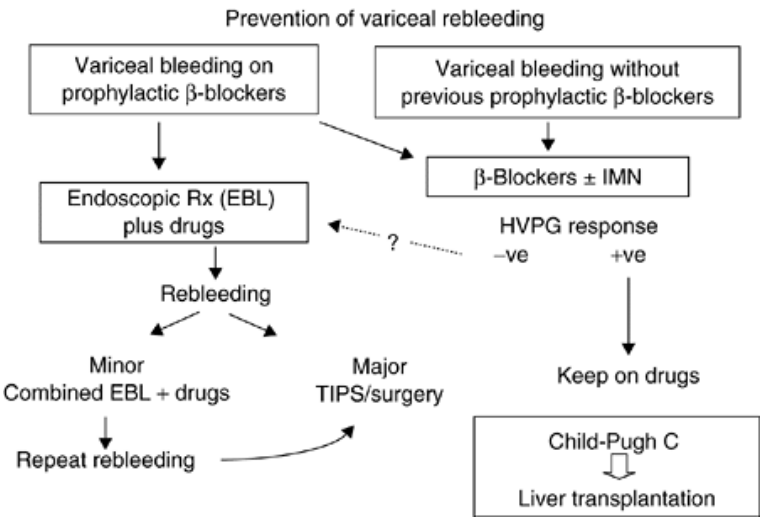
The recommendations for prevention of variceal rebleeding are as follows (Fig. 15.38):

- Patients surviving a bleeding episode should be treated with nonselective β-blockers if they were previously untreated. Those who are intolerant to or have contraindications to β-blockers should be treated with endoscopic therapy. Endoscopic band ligation of esophageal varices is the first choice of endoscopic procedure.
- The combination of β-blockers and endoscopic band ligation is advisable in patients who bleed with β-blockers.
- Whenever possible, the hemodynamic effect of pharmacologic therapy should be monitored. If a reduction of HVPG greater than 20% or below 12 mm Hg is not achieved, IMN or endoscopic therapy may be added.
- In case medical therapy fails, the patients should receive TIPS or derivative surgery. TIPS or surgical shunts (DSRS or 8-mm H-graft shunt) are effective for Child-Pugh class A/B cirrhosis. In nonsurgical candidates TIPS is the only option. PTFE-covered stents improves the results of TIPS.

Treatment of Gastric Varices

There are no specific measures for the prevention of first bleeding from gastric varices. It is conceivable

that the results of pharmacologic therapy are similar as those achieved in the prevention of first bleeding from esophageal varices. Therefore, nonselective β-blockers should be given to patients with large gastric varices to prevent the first bleeding episode.



▪ **Figure 15.38** Schematic representation of the recommended treatment approach to prevent variceal rebleeding. Patients who experience the index bleeding while on prophylactic β-blockers are best treated with a combination of endoscopic band ligation (EBL) and continued drug therapy. Alternatively, as in patients who have not previously taken prophylactic β-blockers, rebleeding can be prevented with pharmacologic therapy alone (β-blockers with or without isosorbide-5-mononitrate [IMN]). In this case, the change in the hepatic vein pressure gradient (HVPG) should be assessed. If it is adequate (reduction in the HVPG by at least 20% of the baseline values or to below 12 mm Hg), continued drug therapy is the best treatment option. Patients failing to show such a response are at higher risk for rebleeding and may benefit from the addition of EBL. Another episode of rebleeding calls for a shift of therapy to TIPS or surgical decompression. All Child-Pugh class C patients should be considered for liver transplantation, irrespective of their response to therapy. Rx, treatment.

The optimal treatment of acute gastric variceal bleeding is not well defined because of the relatively low incidence of this condition. The usual initial treatment is a vasoactive drug(s). Balloon tamponade, with the Linton-Nachlas tube, has been used with limited success (199,278). The tissue adhesive isobutyl-2-cyanoacrylate (Bucrylate), mixed with lipiodol has been found to be efficacious and superior to ethanolamine in nonrandomized studies, achieving hemostasis in 90% of patients (279). In a recent RCT, endoscopic obturation using cyanoacrylate proved more effective and safer than band ligation in the management of bleeding gastric varices (280). However, cerebral embolism has been reported with the tissue adhesives, and interest is therefore focused on thrombin, which provides good hemostasis. Another randomized trial comparing *N*-butyl-2-cyanoacrylate with sclerotherapy in 37 patients had a subset of 17 patients with actively bleeding gastric varices (14). Nonsignificant trends in favor of tissue adhesive were also seen in this small group, and variceal obliteration was significantly more common in the overall group (100% vs. 44%).

Because the rebleeding rate after endoscopic treatment is high, it is recommended that an early decision should be made for TIPS or surgery in patients rebleeding from gastric varices. Salvage TIPS is very effective, with more than 90% success rate for initial hemostasis and an early rebleeding rate below 20%, often from nonvariceal sources, for example, sclerosis ulcers (281).

No specific measures have been studied for the prevention of recurrent bleeding. In clinical practice, nonselective β-blockers are used as first-line therapy. TIPS, shunt

surgery, or variceal obliteration are recommended in failures of pharmacologic treatment (282).

Treatment of Portal Hypertensive Gastropathy and Gastric Antral Vascular Ectasia

There is no indication supporting the primary prophylaxis of bleeding from PHG.

Acute bleeding from PHG should first be treated with the same vasoactive drugs as those for variceal bleeding, although there are no RCTs specifically designed for PHG (283,284,285). Oral propranolol may be used in hemodynamically stable patients (157), starting at 40 mg/day in two divided doses; the dosage may be then titrated up to the maximum tolerated amount.

Recurrent bleeding from PHG should be prevented using nonselective β-blockers, at the same dosage as that used for treating esophageal varices (158). Adequate iron supplementation may be useful to prevent or correct chronic iron-deficient anemia in patients with severe PHG (158). TIPS may be considered as an alternative therapy for the rare patient who has repeated

severe bleeding from PHG despite pharmacologic therapy (282,286). Portal decompressive surgery is reserved for those who are not candidates for orthotopic liver transplantation.

Patients bleeding from *GAVE* may benefit from endoscopic ablation by argon plasma coagulation, neodymium:yttrium-aluminum-garnet (Nd:YAG) laser, or heater probe (282). There is no proof that TIPS and β-blockers are effective for the prevention of recurrent bleeding from GAVE. For select patients with severe recurrent bleeding or uncontrollable acute bleeding from GAVE, an antrectomy with Billroth I anastomosis may be considered (285).

Acknowledgments

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Chapter 16

Surgical Management of Portal Hypertension

J. Michael Henderson

Key Concepts

- Liver transplantation has significantly improved outcome among Child-Pugh class C patients with variceal bleeding.
- Evaluation of patients with portal hypertension being considered for surgery requires endoscopy, vascular imaging, and assessment of the liver disease.
- Primary therapy for variceal bleeding is with endoscopic and pharmacologic modalities.
- Variceal decompression is indicated for patients who fail primary therapy or who cannot undergo primary therapy. Decompression is performed with a transjugular intrahepatic portosystemic or surgical shunt.
- All surgical shunts—total, partial, or selective—control variceal bleeding in more than 90% of patients.
- The occurrence of encephalopathy and liver failure after insertion of a shunt depends on the extent of liver disease and loss of portal perfusion.
- Devascularization procedures are a surgical alternative in the management of variceal bleeding in patients who cannot undergo shunting.

History

Surgical management of portal hypertension was initiated in the late 19th century when Nicolai Eck first performed end-to-side portacaval shunts in dogs (the Eck fistula). Pavlov, more famous for his studies in gastric physiology, used this animal model of shunting all portal flow

away from the liver to describe the portaprival syndrome characterized by meat intoxication (encephalopathy), progressive muscle wasting, and inanition. In the early 1900s, Vidal performed the first portacaval shunt in humans, and Drummond, Morrison, and Talma developed surgeries for portal hypertension in attempts to control variceal bleeding or manage ascites. Banti popularized splenectomy on the basis of his belief that splenomegaly was etiologic in portal hypertension. None had long-term success, but their ideas were the forerunners of much of what followed later (1).

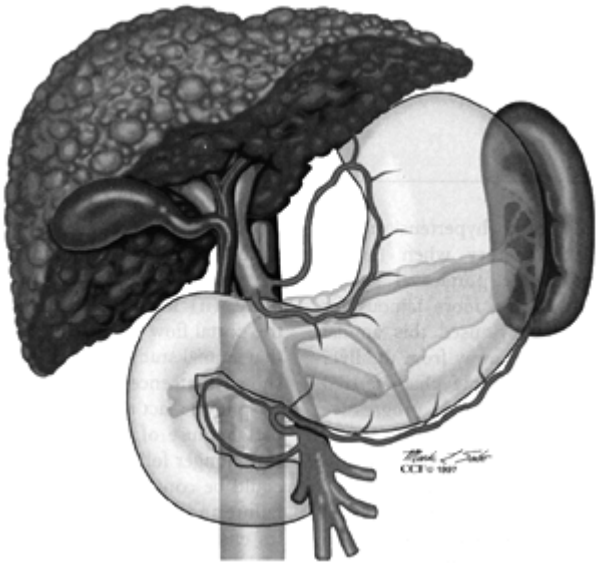
In the 1940s Whipple and the Columbia group reintroduced decompressive shunts for the management of variceal bleeding. His rationale was (i) the recognition that variceal bleeding was not controlled by splenectomy, (ii) that in patients with advanced cirrhosis the portal flow often reduced spontaneously, and (iii) that animal studies with dietary protein restriction had shown that encephalopathy could be controlled (2). Despite the fact that initial results were good, in large part because of the attention to detail and technical skill, longer follow-up showed that although bleeding could be controlled, shunting led to accelerated liver failure and encephalopathy. Randomized clinical studies ensued first with prophylactic shunts in patients with varices that had not bled and subsequently in patients after an initial variceal bleed. These trials in

the 1960s and 1970s showed no significant advantage in patient survival of portosystemic shunts over nonsurgical therapy. The mode of death changed from bleeding to liver failure (3).

In the 1970s and 1980s, surgeons remained active in the field of portal hypertension. Selective variceal decompression was introduced by Warren et al. (4) in the United States and Inokuchi (5) in Japan. They documented that varices could be selectively decompressed with good control of bleeding, maintenance of portal perfusion of the liver, and a lower rate of encephalopathy. Devascularization procedures became more extensive, largely popularized by Sugiura and Futagawa (6) in Japan and Hassab (7) in Egypt. The 1970s also saw the reintroduction of endoscopic sclerotherapy, initially by three surgeons, Johnston, Terblanche, and Paquet. Although initially performed with a rigid esophagoscope, sclerotherapy rapidly moved to flexible endoscopy and was performed by gastroenterologists. In the 1980s the success of sclerotherapy in treating acute bleeding and as first-line treatment to prevent rebleeding diminished the use of shunts (8). Endoscopic therapy evolved with banding replacing injection sclerotherapy in the 1990s (9).

The late 1980s and 1990s saw two other major changes in the treatment of portal hypertension. Liver transplantation became widely used, with excellent outcomes for patients with end-stage liver disease

(10). Second, transjugular intrahepatic portosystemic shunt (TIPS) was introduced in the 1990s (11) and has led to renewed interest in decompressive shunts in the care of patients with adequate liver function who bleed despite endoscopic and pharmacologic therapy but do not need liver transplantation.



▪ **Figure 16.1** Portal venous anatomy. The portal vein is formed by the union of the superior mesenteric and splenic veins behind the neck of the pancreas. The main tributaries are the inferior mesenteric, left and right gastric, and gastroepiploic veins.

Anatomy

Portal venous anatomy is remarkably consistent, considering the complexity of its embryologic development (Fig. 16.1). The portal vein is formed behind the neck of the pancreas by the confluence of the superior mesenteric and splenic veins. The portal vein is normally 10 to 12 mm in diameter but can enlarge up to 20 mm in portal hypertension. It follows a consistent course in the free edge of the gastrohepatic ligament to the porta hepatis, where it divides into right and left branches. The precise sites of entry of other feeding tributaries to this system, which are of clinical relevance in portal hypertension, are more variable. The inferior mesenteric vein enters the splenic vein in approximately two thirds of persons and the superior mesenteric vein in one third. It always serves as an outflow from the portal vein in portal hypertension. The left gastric or coronary vein enters the portal vein in approximately two thirds of persons and the splenic vein in the other one third. The left gastric vein varies considerably in size and may be the major feeding vein of

gastroesophageal varices.

The umbilical vein is constant in its communication with the left branch of the portal vein, and recanalization of this vessel in portal hypertension may open it to a significant size. An understanding of the pattern of tributaries to the portal vein is important to the surgeon both for liver transplantation and for shunts.

The hepatic arterial blood supply is highly variable, and anomalies are of clinical significance to transplantation surgeons, particularly during donor hepatectomy. The normal arterial anatomy is a common hepatic artery arising from the celiac axis and giving rise to right and left hepatic arteries after the gastroduodenal artery leaves the common hepatic trunk. Approximately 20% of persons have an anomalous right accessory or replaced hepatic artery arising from the superior mesenteric artery. The incidence is similar for an accessory or replaced left hepatic artery, which arises from the left gastric artery. These two anomalies can coexist.

The segmental anatomy of the liver subdivides eight segments according to their own major hepatic arterial and portal venous inflow and hepatic venous drainage. This subdivision of the liver is of particular importance in liver resection or for reduced-size liver transplantations. The physiologic functional unit of the liver is the liver lobule, and at this level the hepatic artery and portal venous blood mix, traverse the sinusoids, and drain through the central veins. The hepatic veins are consistent, with the right, middle, and left draining the segmental anatomy of the liver.

The major pathologic changes of the portal venous anatomy in portal hypertension are at the gastroesophageal junction. Reevaluation with radiologic, ultrasound, and corrosion cast studies has shown that the submucosal and periesophageal veins communicate around the gastroesophageal junction through perforating vessels and align in a consistent pattern with the palisades that run in the submucosa in the distal 2 cm portion of the esophagus. This is the most common site of variceal bleeding.

Pathophysiology

Normal portal venous pressure is 5 to 8 mm Hg, with a portal flow of 1 to 1.5 L/minute. The portal vein is a passive conduit that carries blood from the gastrointestinal tract to the liver. Total liver blood flow is regulated by intrinsic and extrinsic mechanisms, with alterations in portal flow causing a reciprocal increase or decrease in hepatic arterial flow.

Portal hypertension is present when portal pressure exceeds 8 mm Hg, and the risk of variceal bleeding occurs at pressures greater than 12

mm Hg. The pathophysiologic sequence of the events in portal hypertension has been defined in animal models (12). The initial resistance to portal venous flow is followed by the development of collateral vessels from the hypertensive bed to the systemic circulation. This is followed by increased plasma volume, development of a hyperdynamic systemic circulation, and marked splanchnic hyperemia, which contributes to the increased portal flow and pressure.

Evaluation

When the surgeon is asked to evaluate a patient with portal hypertension, the focus is on issues pertinent to surgical intervention. A hepatologist has usually evaluated and managed the patient's liver disease and its complications already, and the current status should be defined. The main points of emphasis to the surgeon are the following:

1. Does the patient have decompensated cirrhosis? If this is the case, the only surgical option is liver transplantation.
2. Is the patient bleeding from varices? Have the varices reached a point where they cannot be adequately managed with first-line therapy? First-line management of variceal bleeding is endoscopic and pharmacologic therapy. However, 20% to 30% of patients have rebleeding and need additional therapy. Evaluation is accomplished with endoscopy and vascular imaging. Endoscopy may show varices that cannot be obliterated in the esophagus, gastric varices, or portal gastropathy as the site of bleeding. Doppler ultrasound should be used to show patency of and flow directions in the major veins, but if a decision is being made to perform decompression, angiography is usually needed to obtain details about the site of origin of collateral vessels.
3. Is the patient a suitable candidate for shunt surgery who is likely to survive the surgical procedure? This is primarily assessed by Child-Pugh classification (Table 16.1). Child-Pugh class A patients are surgical candidates. If the patients have no

ascites or encephalopathy, their bilirubin level is less than 3 mg/dL, and the albumin level is greater than 3 g/dL, with an international normalized ratio less than 1.5, the surgeon can operate on them with the expectation of a reasonable outcome. Child-Pugh class B patients may be either class B improving toward class A or class B moving toward class C. In the care of Child-Pugh class C patients, the only realistic surgery is liver transplantation. The role of Model for End-Stage Liver Disease (MELD) scoring (Table 16.2) in assessing patients for shunt surgery has not been fully defined to the same extent as that

used for transplantation.

Table 16.1. Child-Pugh Grading of Severity of Liver Disease

Clinical and laboratory measurement	Patient score for increasing abnormality		
	1	2	3
Encephalopathy (grade)	None	1 or 2	3 or 4
Ascites	None	Mild	Moderate
Bilirubin (mg/dL)	1–2	2.1–3	≥3.1
Albumin (g/dL)	≥3.5	2.8–3.5	≤2.7
Prothrombin time (increase, s)	1–4	4.1–6	≥6.1

Child-Pugh grade is A when total score is 5–6, B when total score is 7–9, and C when total score is 10–15.

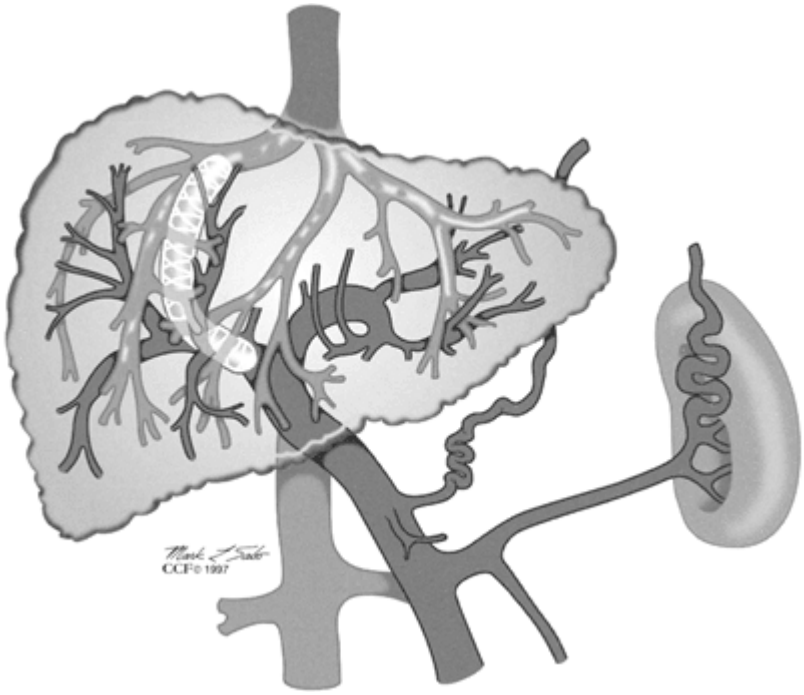
Table 16.2. Model for End-Stage Liver Disease (Meld) Score

Score = 0.957 $\log_e$ creatinine (mg/dL) + 0.378 $\log_e$ bilirubin (mg/dL) + 1.120 $\log_e$ INR
INR, international normalized ratio.

Portal Decompression

Transjugular Intrahepatic Portosystemic Shunt

TIPS emerged in the 1990s as an alternative to surgical decompression (Fig. 16.2) and is currently the most widely used shunt. This shunt, which is addressed in Chapter 15, must, however, also be considered when discussing the site for surgical decompression. Although a TIPS is placed more easily than a surgical shunt, it currently has the disadvantage of high rates of stenosis and thrombosis, leading to rebleeding within 2 years in approximately 20% of patients (13,14). The introduction of polytetrafluoroethylene covered stents has lowered the dysfunction rates, and in a European multicenter trial it reduced rebleeding to 13% at 2 years (15). However, the inability to identify "dysfunction" reliably without repeat shunt catheterization and pressure measurement adds to the complexity of follow-up and cost of TIPS. All patients need an intensive follow-up protocol after TIPS placement. The encephalopathy rate after TIPS appears to be similar to that after total shunt placement (13,16). TIPS is a good treatment for patients with portal hypertension, variceal bleeding, and ascites who are awaiting liver transplantation when bleeding is not controlled with endoscopic therapy (13). The role of TIPS in the care of lower-risk patients who may either have a longer bridge to transplantation or not need transplantation is addressed in subsequent text.



▪ **Figure 16.2** Transjugular intrahepatic portosystemic shunt. A track developed between a major hepatic vein and the portal vein is dilated and stented. This is a side-to-side portal systemic shunt, either total or partial.

Surgical Methods

Three surgical methods are used in the management of portal hypertension:

1. Decompressive shunts
2. Devascularization procedures
3. Liver transplantation

There is a role for each of these operative procedures, and this section discusses the goals and outcome of these procedures.

Decompressive Surgical Shunts

Decompressive surgical shunts fall into three groups:

1. Total portosystemic shunts that decompress all portal hypertension

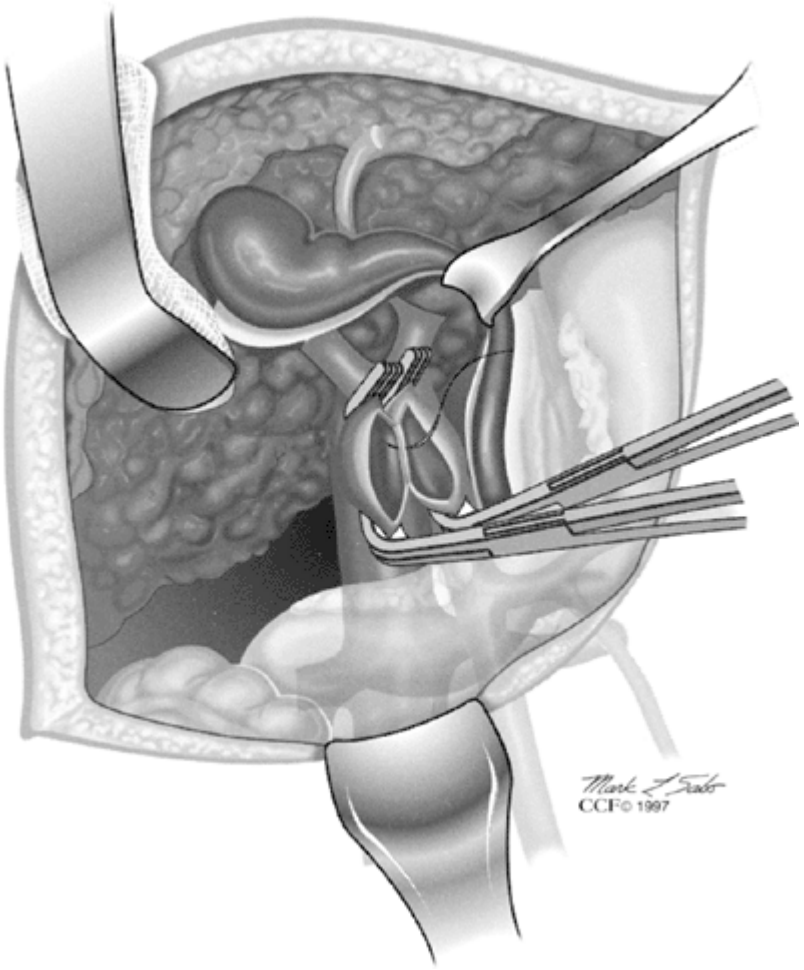
2. Partial portosystemic shunts that reduce portal hypertension to approximately 12 mm Hg
3. Selective shunts that decompress gastroesophageal varices but maintain portal hypertension

Total portosystemic shunts

In the end-to-side portacaval shunt (Eck fistula) procedure, the liver end of the portal vein is ligated and the splanchnic end is anastomosed to the vena cava. With this shunt, the hepatic sinusoids maintain their hypertension; therefore, this surgery does not relieve ascites. Hence, there are currently almost no indications for this surgery at present.

By contrast, the second group of total shunts are side-to-side shunts, all of which decompress the portal hypertension both in the splanchnic bed and in the liver (Fig. 16.3). These procedures still have some proponents. The common factor to these shunts is that they are 10 mm or more in diameter and include side-to-side portacaval, mesocaval, and central splenorenal shunts. They may either be direct vein-to-vein anastomoses or incorporate prosthetic material. The pathophysiology of these shunts is associated with the portal vein acting as an outflow tract from the obstructed sinusoids, with reversal of blood flow in the portal vein to the low-pressure shunt. Side-to-side shunts are excellent for controlling variceal bleeding and ascites but deprive the liver of prograde portal flow and increase the risks of progressive liver failure and encephalopathy (17,18,19). The other major disadvantage of these shunts is that when prosthetic material is used as an interposition graft, there is increased risk of thrombosis (20).

The indications for side-to-side shunts are at present relatively limited. A patient with massive continued bleeding who also has ascites can be treated with a side-to-side shunt, although TIPS achieves the same goal without surgery. The strongest advocates of emergency side-to-side portacaval shunts are Orloff et al. who in 1995 reported an extensive series with excellent outcome in a largely alcoholic group of patients (17). The other indication for this type of side-to-side shunt is acute Budd-Chiari syndrome because it allows decompression of the obstructed sinusoids and halts ongoing hepatocellular necrosis (21).



▪ **Figure 16.3** Side-to-side portacaval shunt. The portal vein and inferior vena cava must be mobilized sufficiently to allow them to be opposed for anastomosis. If the distance between the veins is too great, graft interposition may be needed.

Partial portosystemic shunts

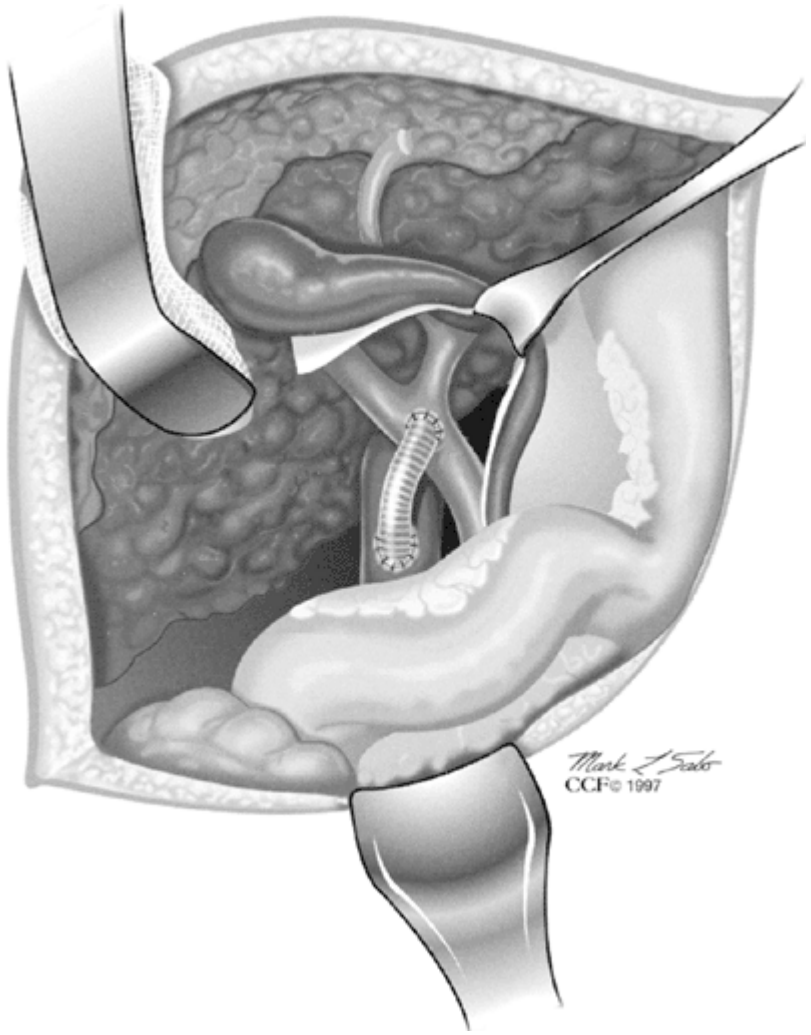
Partial portosystemic shunts can be achieved by reducing side-to-side portosystemic shunt diameter to 8 mm. Data show that at this size portal flow is maintained in 80% of patients, and portal hypertension is reduced to 12 mm Hg (22). This interposition portacaval shunt is illustrated in Figure 16.4. Attention to detail in the execution of this graft is critical to minimize the risk of thrombosis.

Outcomes with partial shunts documented from two prospective, randomized controlled trials indicate control of bleeding in 90% of patients (23,24). The maintenance of some portal flow has been associated with a lower incidence of encephalopathy and liver failure than that with total portosystemic shunting. Other groups have

advocated the use of a limited-size mesocaval shunt in a similar way and have presented data in support of this concept, although not from randomized, controlled studies (25,26).

One randomized trial has compared the 8-mm portacaval surgical shunt to TIPS. In this "all-comers" study, 50% of patients had Child-Pugh C cirrhosis and 63% of the patients had alcoholic liver disease. There was

significantly better control of bleeding and lower need for transplantation in the surgical shunt group compared to the TIPS group—survival was not significantly different (24).



▪ **Figure 16.4** Partial portosystemic shunt. Interposition of an 8-mm expanded polytetrafluoroethylene (Gore-Tex) graft between the portal vein and inferior vena cava reduces portal hypertension to 12 mm Hg and allows continued portal perfusion in 80% of patients.

Selective shunts

In selective variceal decompression, a different pathophysiologic concept is applied for the control of variceal bleeding. Varices are selectively decompressed, usually by a distal splenorenal shunt (DSRS) through the short gastric veins, spleen, and splenic vein to the left renal vein (4). Portal hypertension is maintained in the splanchnic and portal venous system to maintain prograde portal flow to the cirrhotic liver. This surgery is illustrated in Figure 16.5 and has been the most widely used surgical shunt worldwide over the last 20 years. This surgery should be part of the repertoire of liver transplantation surgeons for patients who have refractory bleeding but still have good liver function.

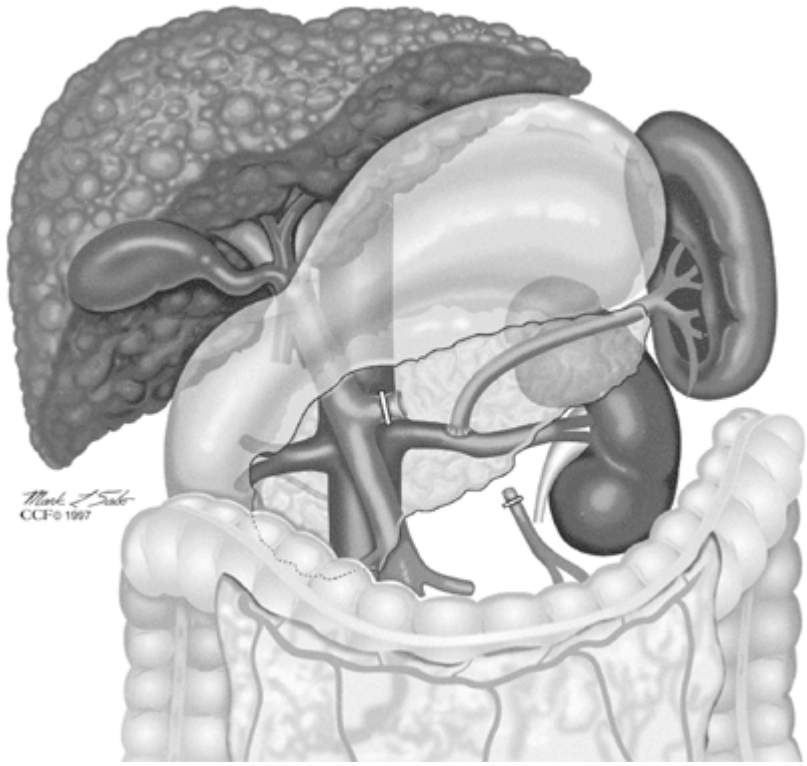
Long-term patency of DSRS is excellent with the vein-to-vein anastomosis, and bleeding is controlled in more than 90% of patients (19,27,28,29,30,31,32,33). The highest risk time for rebleeding is in the first 4 to 6 weeks while the short gastric and renal veins accommodate the increased flow from the enlarged spleen and varices (34). Maintenance of portal perfusion is achieved in 90% of patients in the short term. Long-term maintenance of portal perfusion has proved excellent in nonalcoholic patients, but 50% of alcoholic patients lose portal perfusion unless splenopancreatic disconnection is performed. This has resulted in maintenance of portal flow in 84% of patients with alcoholic cirrhosis (35).

Randomized controlled trials in which DSRS was compared with total shunts have shown equivalent control of bleeding (36). Although three of these six studies have shown lower incidence of encephalopathy after insertion of a DSRS, the others have not. These other studies were conducted with a predominantly alcoholic (83%) population, which may be a factor in the failure to show significant advantage for DSRS.

Four randomized trials have been conducted in which DSRS was compared with endoscopic sclerotherapy (37). In these studies, use of a DSRS resulted in significantly better control of variceal bleeding. The rate of encephalopathy was not significantly different between sclerotherapy and shunt groups. This was the strongest evidence that DSRS does not accelerate the rate of encephalopathy in patients with cirrhosis. One study showed significantly improved outcome among patients initially randomized to sclerotherapy, one third of whom needed subsequent surgical salvage because of recurrent variceal bleeding (38). Another trial showed a significantly better outcome in patients initially randomized to DSRS (39). This difference was predominantly due to the failure to surgically rescue patients in the sclerotherapy group who have rebleeding because they were often geographically remote from the primary managing center. The other

two trials showed no significant difference in survival.

There is much interest in the comparative role of DSRS and TIPS. Several studies have looked at this (a) in a nonrandomized manner (40), in which the superiority of DSRS was demonstrated; (b) in relation to subsequent likely liver transplantation (41); and (c) in a decision analysis evaluation (42), which favored DSRS over TIPS in a cost analysis. A multicenter randomized trial has compared DSRS to TIPS in Child-Pugh class A and B patients who rebled through pharmacologic and endoscopic therapy (43). Sixty percent of the patients entered had alcoholic liver disease. At a median follow-up of 42 months, there was no significant difference in control of bleeding (DSRS 94%, TIPS 89%), time to first encephalopathy event (DSRS, 1 year 20%, 5 years 50%; TIPS, 1 year 22%, 5 years 50%), or survival (DSRS 1 year 85%, 5 years 62%: TIPS 1 year 90%, 5 years 61%). However, the TIPS patients required significantly ($P < 0.001$) more reinterventions (83%) compared to DSRS patients (11%). This emphasizes the need for careful protocol follow-up for TIPS patients—including shunt catheterization and pressure measurements—to achieve these results.



▪ **Figure 16.5** Distal splenorenal shunt. Varices are selectively decompressed through the short gastric veins, spleen, and splenic vein to the left renal vein. Portal hypertension is maintained in the superior mesenteric and portal veins to keep portal flow to the

liver.

Devascularization Procedures

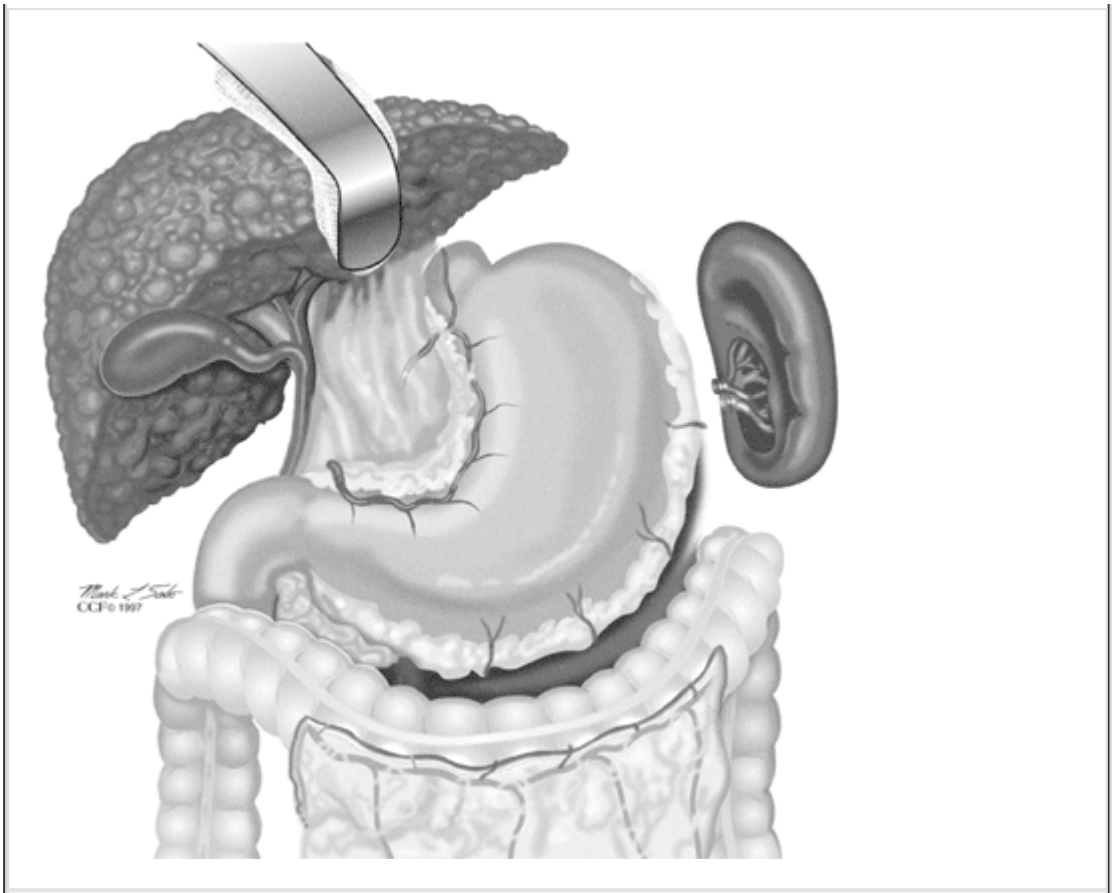
Devascularization procedures have the components of splenectomy, gastric and esophageal devascularization, and in some situations, esophageal transection (Fig. 16.6). The advantage of these procedures is that they maintain portal hypertension and portal flow to the cirrhotic liver and do not accelerate liver failure or encephalopathy. The disadvantage is that they have a higher rate of rebleeding, which probably depends on the extent of the surgical procedure. Sugiura et al. devascularized from the pylorus to the inferior pulmonary veins using both thoracotomy and laparotomy to achieve an extensive procedure with a low rate of rebleeding (6,44). Lesser procedures using only a transabdominal approach, as performed in Europe and the United States, have rebleeding rates in the range of 20% to 40% (45). In patients who have extensive portal venous thrombosis and in whom no vessels can be shunted, devascularization procedures are the only surgical option. When these patients do not have bleeding controlled by pharmacologic and endoscopic therapy, extensive devascularization may significantly reduce the risk of rebleeding. This is the major indication for devascularization procedures.

Liver Transplantation

Liver transplantation has dramatically altered the outcome for patients with advanced liver disease, portal hypertension, and variceal bleeding. It is the one therapy that has significantly improved survival of patients with bleeding varices and Child-Pugh class C cirrhosis. The indication for liver transplantation, however, is end-stage liver disease rather than variceal bleeding.

Liver transplantation both restores hepatic function and relieves portal hypertension, making it the most effective shunt in the management of variceal bleeding. However, supply and demand dictate that transplantation cannot be the therapy for all patients with cirrhosis who have variceal bleeding. The evolution of national organ allocation standards for livers has had an impact on who receives the transplant. The sickest patients from the perspective of the severity of liver disease receive priority, and variceal bleeding per se does not increase priority. This appropriately drives the need

to use the other modalities discussed in this chapter to treat variceal bleeding in patients who still have adequate liver function.



▪ **Figure 16.6** Devascularization procedures have the components of splenectomy and devascularization of the greater and lesser curves of the stomach, as well as the distal 7 cm of the esophagus.

The outcome of liver transplantation has dramatically improved in the last decade. Currently, patients can anticipate an initial 6-month mortality of approximately 10%, with continuing long-term risk of 2% to 5% per year for death or major morbidity. The major risk factors facing transplantation patients are recurrent disease, especially hepatitis, chronic rejection, and immunosuppression-related infection.

In the context of this chapter, for the management of variceal bleeding, liver transplantation is the most viable long-term treatment option for patients with variceal bleeding and end-stage disease. Timing is always a major issue, and with the increasing lengthening of waiting lists it becomes more problematic. Determining the correct "bridge" to transplantation is key, and the least therapy that can be used as a bridge, the better it is for the patient. However, when patients have bleeding refractory to endoscopic therapy, it may be appropriate to decompress the portal hypertension with TIPS. Surgical decompression is also an appropriate bridge for a patient who has a

disease that may not necessitate transplantation for 5 to 10 years.

Management Strategies for Variceal Bleeding

Prophylaxis

There is no indication for surgical intervention as prophylaxis before the first episode of variceal bleeding. Current data indicate that prophylaxis should be with pharmacologic therapy. In some patients at high risk with large varices, endoscopic therapy as prophylaxis against initial variceal bleeding may be indicated. The data on surgical prophylaxis are old, primarily being collected in the 1960s, but still lead to the recommendation of no surgical intervention before an initial episode of bleeding.

Acute Variceal Bleeding

Primary therapy for acute variceal bleeding is endoscopic. Sclerotherapy or banding is done at the time of the initial diagnostic endoscopic procedure. This treatment may be combined with pharmacologic therapy to reduce the risk of early rebleeding. Surgical intervention is rarely indicated for acute variceal bleeding.

More than 90% of patients can be treated with endoscopic therapy. In the small minority of cases in which this therapy fails, balloon tamponade with emergency decompression using TIPS in the next 24 hours is the best approach. The one exception to this view is that of Orloff et al. (17), who continue to advocate emergency portacaval shunting for acute variceal bleeding. The success of their approach is probably based on the patient population they treat and their commitment to early intervention and a program of long-term follow-up evaluation of these patients.

Prevention of Variceal Rebleeding

Surgical intervention is part of an overall program for this group of patients but has been progressively replaced with new options. Initial first-line treatment is with pharmacologic and endoscopic therapies. Seventy percent of patients are successfully treated in this way.

It is for the 30% of patients who have rebleeding through first-line treatment or in whom the varices are not obliterated that decompression should be considered. How should this decompression be achieved? A key decision at this point is whether the patient has end-stage liver disease or a disease that is rapidly approaching this stage. Patients who do have such disease should be fully evaluated for transplantation and moved in this direction. For patients who rebleed through first-line treatment and have adequate and stable liver function, the choice of surgical shunt or TIPS is based on availability,

access to care, and commitment to follow-up. Both are equally efficacious in outcomes.

Reaching decisions in patient care requires early and accurate evaluation. Once a patient's condition has been stabilized after acute variceal bleeding, the patient should undergo full evaluation as outlined earlier. Equally important for patients being treated with first-line therapy is to monitor whether the disease is stable and whether hepatocellular function is deteriorating or improving. Patient education is also key to the care of these patients over the long term so that they understand that there are other treatment options should first-line treatment fail.

New Trends as a Consequence of Improved Control of Variceal Bleeding

The advances and improvements in the control of variceal bleeding in the last two decades have led to the increased recognition of other complications of cirrhosis, which were previously uncommon. Two that are clinically important, and should be considered in long-term management of these patients, are hepatocellular carcinoma (HCC) and the pulmonary complications of chronic liver disease.

HCC is increasing in frequency for many reasons. Improved management of variceal bleeding, with overall improved survival of an "at-risk" population, is one contributing factor. Prospective evaluations of such patients by the Barcelona Group gave a 14% to 21% risk of HCC at 5 years, but their data do not indicate that shunts increase that risk (46).

The pulmonary complications of cirrhosis are the hepatopulmonary syndromes (HPSs) and portopulmonary hypertension (PPH). HPS is seen in up to 15% of patients with cirrhosis (47), whereas PPH is less common. The role of portal–systemic shunting in the development of either of these has been widely debated. The pathophysiology of HPS is a nitric oxide–mediated pulmonary vasculature vasodilatation: How far this is triggered by mediators from the damaged liver or through portal systemic shunting is not fully defined (47). A larger multicenter review of both these complications in patients considered for transplantation did not implicate surgical or radiologic shunts as etiologies in the patients who were identified (48). The current data leaves open the question of the importance of shunting in these syndromes.

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Chapter 17

Renal Complications and Hepatorenal Syndrome

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Key Concepts

- Renal impairment in most patients with cirrhosis is secondary to functional abnormalities that occur in response to a severe splanchnic arterial vasodilatation which triggers an intense homeostatic neurohumoral response causing sodium retention and solute-free water retention and, finally, severe renal vasoconstriction.
- Sodium retention is the first and most common functional renal abnormality in patients with cirrhosis and plays a fundamental role in the formation of ascites and edema. The total amount of extracellular fluid accumulated as ascites or edema depends on the balance between sodium intake and excretion.
- In the natural history of cirrhosis and ascites, in time, patients develop an impairment in the renal handling of solute-free water and retain water in excess of sodium. Those with a marked impairment in solute-free water excretion develop dilutional hyponatremia, a condition that carries a poor prognosis in patients with cirrhosis and ascites.
- Treatment of spontaneous dilutional hyponatremia with V2 receptor antagonists in patients having cirrhosis and ascites seems to be a promising new therapy that enhances solute-free water excretion and raises serum sodium levels in this condition.
- Hepatorenal syndrome (HRS) is the end of the spectrum of functional renal abnormalities caused by a severe vasoconstriction of the renal circulation. Patients with marked sodium and water retention are at high risk of developing HRS.
- There are two types of HRS; type 1 is an acute and rapidly progressive form with a very poor prognosis and type 2 is a more stable form with a slightly better prognosis.
- The short-term mortality rate in patients with HRS is very high. The type of HRS and the Model for End-stage Liver Disease (MELD) score are predictive factors of survival. Transplantation candidates should undergo transplantation with a high priority.
- HRS that develops in patients with cirrhosis after spontaneous bacterial

peritonitis (SBP) or in the setting of acute alcoholic hepatitis can be prevented effectively. The administration of intravenous albumin together with antibiotic therapy in the former condition, and oral pentoxifylline in the latter prevent HRS.

- The treatment of HRS should be aimed at reversing the intense splanchnic arteriolar vasodilatation with splanchnic vasoconstrictors and plasma expansion as a bridge to liver transplantation. The use of transjugular intrahepatic portosystemic shunt also appears to be effective in select cases.

Table 17.1. Functional Renal Abnormalities in Cirrhosis

Abnormality	Clinical consequence
Sodium retention	Ascites and edema
Solute-free water retention	Spontaneous dilutional hyponatremia
Renal vasoconstriction	Hepatorenal syndrome

In the natural history of cirrhosis, a progressive derangement of renal function, which is functional in nature, leads to an inability to maintain the extracellular fluid volume within normal limits. This abnormal extracellular fluid volume regulation is due to alterations in the splanchnic and systemic arterial circulation that cause functional renal abnormalities, leading to sodium retention. The characteristic and predominant renal function abnormality in cirrhosis is sodium retention and its main clinical consequence is the recurrent accumulation of extracellular fluid as ascites and/or edema. Sodium retention, when severe, is often associated with an impaired ability to eliminate a regular water intake, which may lead to dilutional hyponatremia due to an unbalanced increase in total body water, relative to the total sodium content (i.e., water in excess of sodium). With disease progression a gradual vasoconstriction of the renal circulation usually develops because of circulating vasoconstrictors. This event causes renal hypoperfusion, reduction in glomerular filtration rate (GFR), and eventually renal failure or the so-called hepatorenal syndrome (HRS) (Table 17.1). All these abnormalities of renal function contribute significantly to the high morbidity and mortality, characteristic of cirrhosis.

The mechanisms leading to renal dysfunction in cirrhosis are not completely understood and are still a matter of investigation. Extrarenal and intrarenal vasoactive factors, sodium and water retaining systems, abnormalities in systemic, cardiac and splanchnic hemodynamics, and the diseased liver causing severe portal hypertension and hepatic failure play an important role in the pathophysiology of HRS. This chapter describes the clinical characteristics,

pathogenesis, and treatment of renal complications in patients with cirrhosis.

Clinical Features

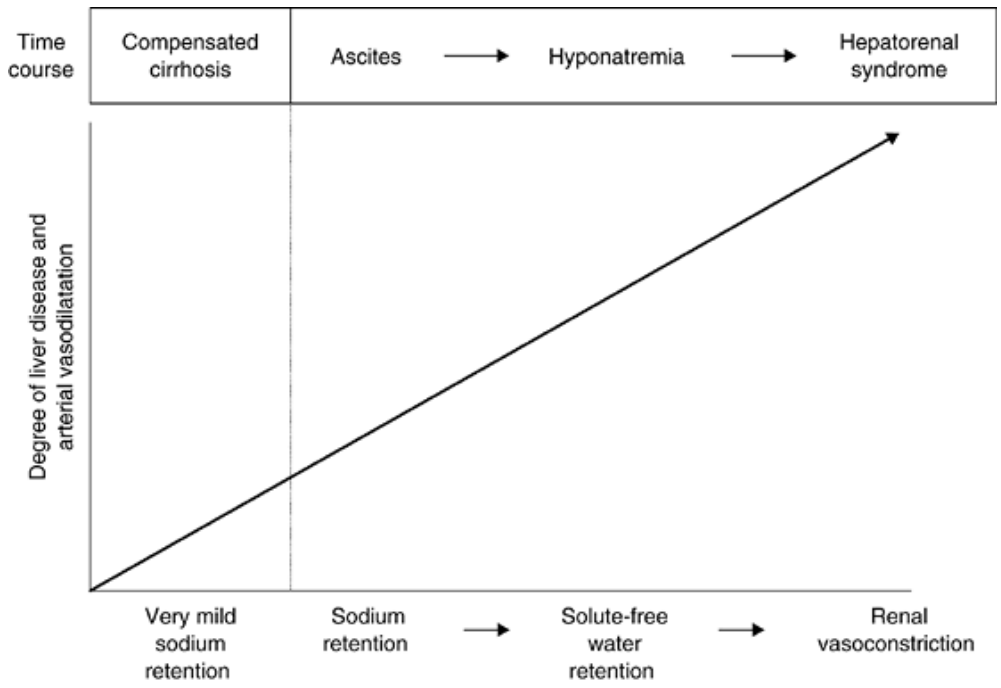
Sodium Retention and Ascites/Edema

Sodium retention is the most common abnormality of renal function in patients with cirrhosis and ascites and plays a key role in the pathophysiology of ascites and edema formation (1,2,3). It is the first manifestation of renal impairment these patients develop (Fig. 17.1). The total amount of sodium retained by patients with cirrhosis and the subsequent gain of extracellular fluid is dependent on the balance between sodium intake and excretion. Patients accumulating ascites and edema invariably have a lower amount of sodium excreted in the urine than their dietary intake. However, if they decrease their sodium intake and use diuretics, sodium excretion increases and they lose extracellular fluid and ascites and/or edema decrease. Compensated patients with cirrhosis (no past history of ascites) usually do not retain sodium while consuming a normal diet, but may develop ascites and/or edema when sodium intake is increased (high-sodium diet or administration of intravenous saline solutions) or when they are treated with drugs that increase sodium reabsorption, such as mineralocorticoids or nonsteroidal anti-inflammatory drugs (NSAIDS) (2,3,4,5).

The intensity of sodium retention varies considerably from patient to patient. In baseline conditions (low-sodium diet and without diuretics) some patients have relatively high urinary sodium excretion, whereas in other patients, urinary sodium excretion is very low (Fig. 17.2) (6). The underlying mechanisms leading to this variability are not well understood; however, it seems that they could be related to the amount of circulating sodium-retaining systems (mainly aldosterone), increased sensitivity to these, or an increase in renal tubular sodium transporters regulated by aldosterone (2,7). Most patients who require hospitalization for the treatment of ascites have marked sodium retention with a very low baseline urinary sodium excretion (usually <10 mEq/day). Patients with moderate sodium retention (≥10 mEq/day) have a better response to diuretic therapy than those with marked sodium retention (8). Although sodium retention in cirrhosis is not a fixed and unalterable disorder, in most patients the intensity of the disorder increases over time. However, a subset of patients with marked sodium retention (particularly abstinent alcoholics with cirrhosis) may improve spontaneously and remain without ascites for prolonged periods of time.

The underlying mechanism responsible for renal sodium retention is an increased renal tubular reabsorption of sodium in the proximal and distal tubules (2,9). This occurs even in the presence of a normal or only moderately reduced GFR (10,11). Although numerous mechanisms have been proposed to explain sodium retention in cirrhosis, the two main sodium-retaining systems responsible are the

renin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS). These are activated as a homeostatic response to a circulatory dysfunction (see later). The two final products of the RAAS system, angiotensin II and aldosterone, induce sodium retention by acting in the proximal tubule (12). The SNS also stimulates sodium reabsorption, particularly in the proximal tubule (13).



▪ **Figure 17.1** Schematic representation of the time course of the functional renal abnormalities and the relationship with the underlying liver disease and arterial vasodilatation in patients with cirrhosis.

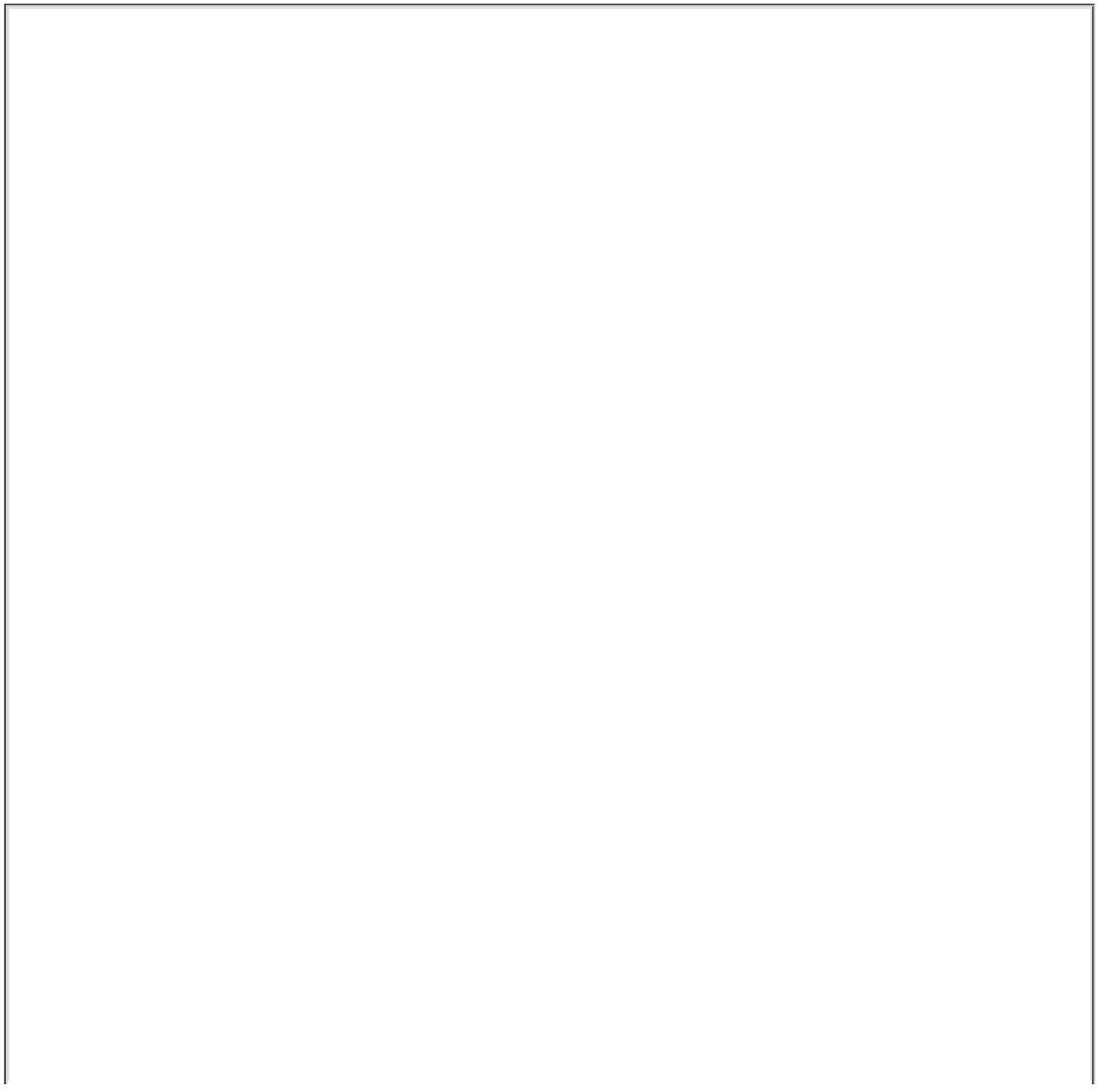
Solute-Free Water Retention and Dilutional Hyponatremia

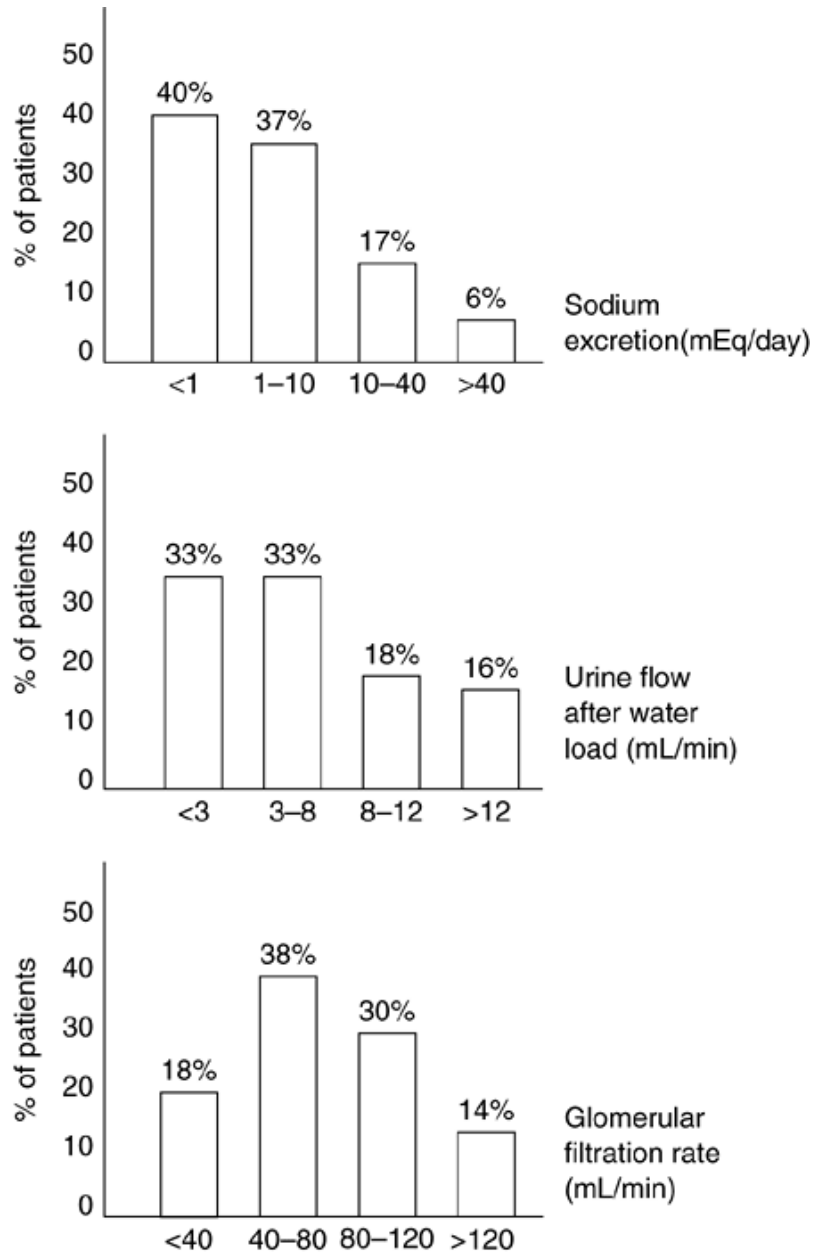
Patients with cirrhosis without ascites usually have normal or only slightly impaired renal water handling. In these patients, total body water, plasma osmolality, and serum sodium concentration are normal and do not develop hyponatremia even in conditions of high fluid intake (3,4,14,15). In contrast, an impairment in the renal capacity to excrete solute-free water is common in patients with cirrhosis and ascites. Approximately 75% of patients with cirrhosis and ascites have an impaired renal water handling, as indicated by an inability to eliminate a water load (20 mL/kg of 5% dextrose)(16). However, the intensity of this disorder is not uniform and varies markedly from patient to patient (Fig. 17.2). In some patients, impaired solute-free water excretion is moderate and can be detected only after the administration of a water load (16). These patients maintain a normal serum sodium concentration as long as their water intake is kept within normal limits. However, they may develop dilutional hyponatremia and hypo-osmolality when water intake is increased (i.e., administration of intravenous fluids in hospitalized patients) (6). Therefore, a normal serum sodium concentration in a patient with cirrhosis and ascites is not synonymous with a normal renal capacity to excrete solute-free water. In other patients, the severity of solute-free water retention is such that they retain most of the water in their diet, causing spontaneous dilutional hyponatremia and hypo-osmolality. Additionally, impairment in solute-free water excretion may be aggravated by administration of diuretics or NSAIDS and large-volume paracentesis without plasma volume expansion (17,18). Dilutional hyponatremia defined as serum sodium level <130 mEq/L in cirrhosis occurs in the presence of marked sodium

retention, with water being retained in excess of sodium, leading to the dilution of body fluids and a fall in serum sodium concentration (19). This indicates that in patients with cirrhosis, sodium retention occurs first and the inability to excrete solute-free water is a later event (Fig. 17.1). Moreover, patients with marked impairment in solute-free water excretion have a high risk of developing HRS during follow-up (20).

The pathogenesis of solute-free water retention in cirrhosis seems to be related to three events: (i) A reduced delivery of filtrate to the ascending limb of the loop of Henle (the diluting segment of the nephron), (ii) a reduced renal synthesis of prostaglandins (PGs), and (iii) an increased secretion of arginine vasopressin (AVP), the antidiuretic hormone (14,21). Among these, AVP is probably the most powerful factor that triggers intense solute-free water retention in cirrhosis with ascites. High levels of AVP in cirrhosis due to nonosmotic hypersecretion of AVP from the neurohypophysis

in response to a reduced effective intravascular volume secondary to vasodilatation in the splanchnic circulation are responsible for water reabsorption in the distal collecting duct of the kidney (see later). The renal synthesis of PGE_2 is increased in patients with cirrhosis to counteract the water-retaining effect of AVP and hence NSAIDs may impair the renal ability to excrete solute-free water in these patients (6,17).





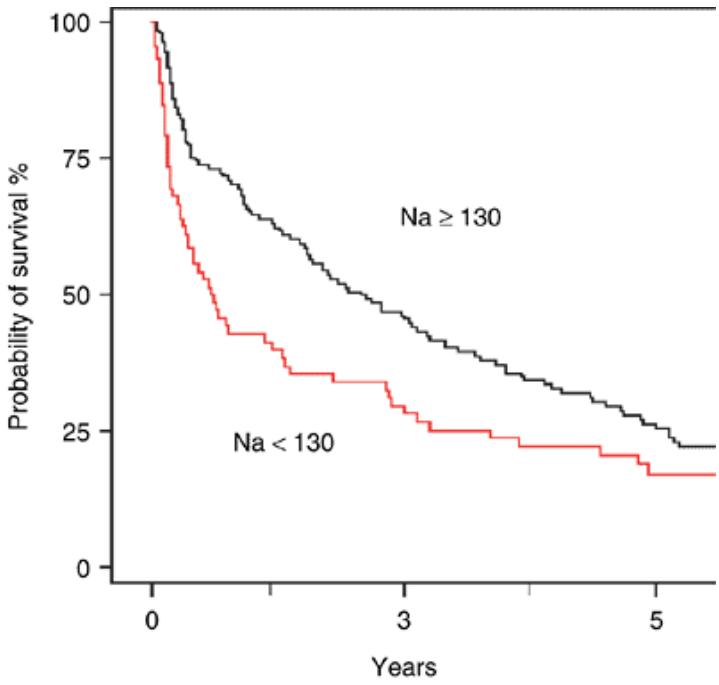
▪ **Figure 17.2** Urinary sodium excretion, diuresis after a water load (20 mL/kg body weight of 5% dextrose IV) and glomerular filtration rate (GFR, insulin clearance) in a series of patients with cirrhosis hospitalized for the treatment of an episode of ascites. All patients were studied after a minimum of 5 days on a 50 mEq/day sodium diet and without diuretic therapy. Values in healthy subjects studied under the same conditions are urine sodium, 40 to 60 mEq/day; diuresis after a water load, 10 to 18 mL/minute; GFR, 110 to 140 mL/minute. Most patients had marked sodium and water retention. Moderate reductions of GFR were present in two thirds of patients, whereas a marked reduction of this parameter was found in 18%. (From Ginès P, Fernández-Esparrach G, Arroyo V, et al. Pathophysiology of ascites. *Semin Liver Dis* 1997;17:175, with permission.)

As stated previously, the clinical consequence of impairment in solute-free water

excretion is the development of hyponatremia. This type of hyponatremia is referred to as *dilutional hyponatremia* because it occurs in the setting of an increased total body water and dilution of extracellular fluid volume. Dilutional hyponatremia associated with sodium retention and increased total body sodium level should be distinguished from true hyponatremia due to sodium depletion that, although less common, may develop in patients with cirrhosis who are maintained on high doses of diuretics causing a negative sodium balance after complete resolution of ascites and edema. The prevalence of spontaneous dilutional hyponatremia in hospitalized patients with cirrhosis and ascites is approximately 30% (22,23). Spontaneous dilutional hyponatremia is also associated with poor survival among patients with cirrhosis and ascites with an estimated 1-year probability of survival of 40% (A. Cárdenas, P. Ginès, unpublished observations, 2003) (Fig. 17.3). Although hyponatremia in cirrhosis and ascites had been recognized as a marker of poor prognosis in the past, it was not considered an important prognostic factor in clinical practice. Only recently it has gained attention as a strong prognostic marker, particularly in patients awaiting liver transplantation, given that several reports indicate that when serum sodium concentration is combined with the Model for End-Stage Liver Disease (MELD) score it improves the prognostic accuracy of MELD in patients listed for orthotopic liver transplantation (24,25,26).

Patients with cirrhosis and ascites usually develop dilutional hyponatremia over several days or weeks. In most patients the degree of hyponatremia is mild, with levels ranging from 125 to 130 mEq/L; nonetheless some patients may have lower values reaching 110 to 125 mEq/L (22). In some patients, hyponatremia is asymptomatic, but in others it may be associated with

clinical symptoms similar to those found in hyponatremia of other etiologies, including nausea, vomiting, apathy, anorexia, headache, difficulty in mental concentration, disorientation, sluggish thinking, and lethargy. Nevertheless, neurologic symptoms related to hyponatremia may be difficult to distinguish from those of hepatic encephalopathy, which may also occur concomitantly with dilutional hyponatremia. Important advances have been made trying to elucidate the role of hyponatremia and its relationship with hepatic encephalopathy. The astrocytes of patients with cirrhosis and hyponatremia have a reduced amount of organic osmolytes (mainly myo-inositol) as measured by magnetic resonance spectroscopy, that may help prevent the development of brain cell edema (27,28). This indicates that organic osmolytes could play a role in cerebral fluid homeostasis in cirrhosis, and in theory they predispose to disturbances in neurologic function. Preliminary studies in patients with cirrhosis and refractory ascites suggest that dilutional hyponatremia is a major predisposing factor to the development of hepatic encephalopathy (29). Further studies in this area are required to establish this possible pathogenic relationship.



▪ **Figure 17.3** Survival of a large group of patients with cirrhosis and ascites with and without dilutional hyponatremia (P. Ginès & A. Cárdenas, *unpublished data*, 2003).

Renal Vasoconstriction and Hepatorenal Syndrome

Renal vasoconstriction leading to decreased renal perfusion is the renal functional abnormality that develops last in patients with cirrhosis and ascites (Fig. 17.1) (2,6). As with the impairment in sodium and solute-free water excretion, the intensity of renal vasoconstriction is variable among patients with ascites (Fig. 17.2). Moderate degrees of renal vasoconstriction are commonly overlooked in clinical practice, because tests used to estimate GFR in the clinical setting have low sensitivity in cirrhosis due to a low endogenous production of creatinine. The existence of a moderate renal vasoconstriction (serum creatinine between 1.2 and 1.5 mg/dL, which corresponds to a median GFR of approximately 50 mL/minute) in patients with cirrhosis and ascites is clinically relevant for several reasons. First, a significant proportion of these patients have refractory ascites, as sodium and water excretion are markedly impaired. Second, moderate renal vasoconstriction predisposes to the development of HRS (20). Finally, mortality is higher in these patients than in those with normal renal perfusion (20,30).

An increased activity of vasoconstrictor factors (mainly plasma renin activity and norepinephrine) and reduced activity of renal vasodilator factors acting on the renal circulation play the most important role in the pathogenesis of HRS because renal vasoconstriction in cirrhosis occurs in the absence of morphologic changes in the kidney (1). The pathogenesis of renal vasoconstriction in cirrhosis is also related to changes in systemic hemodynamics. The most accepted theory considers that renal vasoconstriction is the consequence of the extreme underfilling of the systemic arterial circulation due to marked vasodilatation of the splanchnic circulation, which activates homeostatic vasoconstrictor systems,

whose effect on the kidney vasculature cannot be counterbalanced by either renal or systemic vasodilators (31,32). As a consequence, severe vasoconstriction of renal vessels occurs and HRS ensues. Nonetheless, other reasons causing renal dysfunction in cirrhosis have been suggested. For instance, a role for intrahepatic adenosine causing an increase in portal venous blood flow and triggering a hepatorenal reflex to regulate sodium and water excretion has been recently proposed as a mechanism that may lead to decreased renal perfusion and HRS (33,34). This mechanism by means of increasing sympathetic activity in the kidney probably decreases renal blood flow and GFR (34,35). In humans, the presence of hepatorenal reflex has been suggested with observations of reduced renal blood flow following an increase in portal pressure and renal release of endothelin, suggesting that this vasoconstrictor could perhaps play a role in this reflex (35). Further studies in animals and humans are needed to elucidate the role of hepatorenal reflex in HRS.

The definition of HRS proposed by the International Ascites Club is the most widely accepted and states: "Hepatorenal syndrome is a clinical condition that develops in patients with chronic liver disease and advanced hepatic failure and portal hypertension characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of the endogenous vasoactive systems. In the kidney, there is marked renal vasoconstriction that results in low GFR. There is also vasoconstriction in other vascular territories such as the muscle, spleen and brain. In the splanchnic circulation, there is an intense arteriolar vasodilatation that results in reduction of total systemic vascular resistance and arterial hypotension. A similar syndrome can also develop in the setting of acute liver failure" (32).

The incidence of HRS in patients with cirrhosis hospitalized for ascites is approximately 10% (20). However, the probability of developing HRS in patients with cirrhosis and ascites is 18% at 1 year and increases to 39% at 5 years (20). Two different types of HRS that represent distinct expressions of the same pathogenic mechanism have been defined (Table 17.2) (32). Renal failure in HRS in most cases is associated with oliguria, marked sodium retention, and spontaneous dilutional hyponatremia. Patients with type 1 HRS (serum creatinine >2.5 mg/dL [220 μmol/L]) develop a very rapid and aggressive course of illness, becoming very ill in a matter of days to weeks; these patients usually have signs of advanced liver disease. In nearly half of patients,

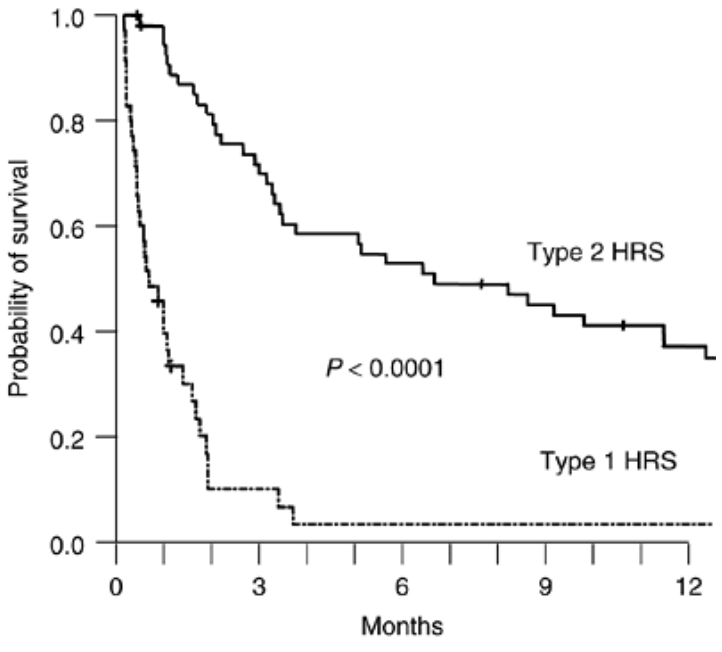
this type of HRS develops spontaneously without any identifiable precipitating factor, whereas in the rest it can occur in close association with systemic bacterial infections such as spontaneous bacterial peritonitis (SBP), surgical operations, and acute alcoholic hepatitis (30,32,36). In addition, large-volume paracentesis (≥5 L) without albumin expansion may precipitate type 1 HRS in approximately 20% of cases (18). This is one of the reasons why intravenous albumin is routinely administered after large-volume paracentesis in patients with cirrhosis and ascites. Bacterial infections in patients with cirrhosis, particularly SBP, are now recognized as the most common precipitating cause of type 1 HRS, which occurs in approximately one third of cases with SBP despite resolution of the infection with intravenous antibiotics (37,38,39). Renal failure occurs in 10% of patients with cirrhosis and gastrointestinal bleeding (40). However, renal failure in this setting occurs mainly in patients who develop hypovolemic shock, suggesting acute tubular necrosis (ATN) and not HRS as the cause of renal failure (40). Although contrast media for radiologic procedures was previously felt to be a frequent precipitant of renal failure in patients with cirrhosis and ascites,

recent data indicates that contrast media in these patients is very infrequently associated with the development of renal failure (41).

Table 17.2. Clinical Types of Hepatorenal Syndrome

Type 1	Rapid and progressive impairment of renal function as defined by a doubling of the initial serum creatinine to a level higher than 2.5 mg/dL (220 μmol/L) or a 50% reduction of the initial 24-h creatinine clearance to a level lower than 20 mL/min in <2 wk
Type 2	Impairment in renal function with serum creatinine >1.5 mg/dL (130μmol/L) that does not meet criteria for type 1

Without treatment, the median survival time of patients with type 1 HRS is approximately 1 month and practically all patients die within 10 to 12 weeks of renal failure onset (20,30,42). The MELD score along with type of HRS have an independent prognostic value for survival (43) (Fig. 17.4). Most patients with type 1 HRS have a MELD score ≥20 (43). In contrast to type 1 HRS, type 2 HRS is characterized by a less severe and stable reduction of GFR (serum creatinine levels are usually <2.0 mg/dL or 177 μmol/L). The main clinical consequence of type 2 HRS is diuretic-resistant ascites due to the combination of intense sodium retention and reduced GFR. As expected, survival is longer in patients with type 2 HRS than in those with type 1 HRS, but is shorter than that of patients with cirrhosis and ascites without renal failure (20,42,43) (Fig 17.4). In patients with type 2 HRS, a MELD score of 20 or more is also associated with a poor outcome (43).



▪ **Figure 17.4** Probability of survival of patients with cirrhosis according to type of hepatorenal syndrome (HRS). (From Alessandria C, Ozdogan O, Guevara M, et al. MELD score and clinical type predict prognosis in hepatorenal syndrome: relevance to liver transplantation. *Hepatology* 2005;41:1282–1289, with permission.)

Predictive factors associated with a greater risk of developing HRS have been described in patients with cirrhosis and ascites (Table 17.3) (20,30). Patients with intense sodium retention (<10 mEq/day), spontaneous dilutional hyponatremia (serum sodium <130 mEq/L), low mean arterial blood pressure (<85 mm Hg), decreased cardiac output (<6.0 L/minute) and increased plasma renin activity, aldosterone, and norepinephrine levels have a high probability of developing HRS (20,30). Other parameters such as the degree of liver failure, as assessed by parameters of liver function (serum bilirubin, albumin, and prothrombin time), the Child-Pugh score, and hepatic venous pressure gradient have not consistently shown to predict the development of type 1 HRS.

There is no specific test or marker for the diagnosis of HRS. The diagnosis of HRS is based on the demonstration of a reduced GFR in the absence of data suggesting other causes of renal failure. The proposed criteria for the diagnosis of HRS are listed in Table 17.4 (32). Low GFR is defined as serum creatinine level greater than 1.5 mg/dL (130 μmol/L) or 24-hour- creatinine clearance lower than 40 mL/minute, without diuretic therapy. Other criteria include the absence of precipitants of renal failure (i.e., volume depletion, shock, bacterial infections, or nephrotoxic drugs), no improvement of renal function following diuretic withdrawal and plasma expansion, no proteinuria, and a normal renal ultrasonogram. Other causes of renal failure in cirrhosis such as prerenal renal failure secondary to volume depletion, ATN, drug-induced nephrotoxicity

(mainly from nonsteroidal anti-inflammatory agents or aminoglycosides), and glomerulonephritis in patients with hepatitis B or C should be excluded before the diagnosis of HRS is made. Most cases of HRS have urine sodium level below 10 mEq/L.

Table 17.3. Parameters Associated with a Higher Risk of Hepatorenal Syndrome Development in Nonazotemic Patients with Cirrhosis and Ascites[a]

Previous episodes of ascites
Poor nutritional status
Moderately increased BUN[b]
Moderately increased serum creatinine[b]
Low serum sodium (serum sodium <130 mEq/L)
Low urinary sodium excretion (urine sodium <10 mEq/L)
High plasma renin activity (>4 ng/mL per h)
Low mean arterial pressure (<85 mm Hg)
Reduced solute-free water excretion after water load (<3 mL/min)
Increased plasma norepinephrine (>500 pg/mL)

Presence of esophageal varices
Model for End-Stage Liver Disease score

[a]All measurements were obtained after a minimum of 5 days on a low-sodium diet and without diuretics.
[b]Blood urea nitrogen and serum creatinine values up to 30 mg/dL and 1.5 mg/dL (130 μmol/L), respectively.
From Ginès A, Escorsell A, Ginès P, et al. Incidence, predictive factors, and prognosis of hepatorenal syndrome in cirrhosis. *Gastroenterology* 1993;105:229–236; Ruiz del Arbol L, Monescillo A, Arocena C, et al. Circulatory function and hepatorenal syndrome in cirrhosis. *Hepatology* 2005;42:439–447.

Table 17.4. Diagnostic Criteria of Hepatorenal Syndrome According to the International Ascites Club[a]

Major criteria

1. Low glomerular filtration rate, as indicated by serum creatinine >1.5 mg/dL (130 μmol/L) or 24-h creatinine clearance <40 mL/min
2. Absence of shock, on-going bacterial infection, fluid losses, and current treatment with nephrotoxic drugs
3. No sustained improvement in renal function (decrease in serum creatinine to 1.5 mg/dL [130 μmol/L] or less or in crease in creatinine clearance to 40 mL/min or more) following diuretic withdrawal and expansion of plasma volume with 1.5 L of a plasma expander
4. Proteinuria <500 mg/d and no ultrasonographic evidence of obstructive uropathy or parenchymal renal disease

Additional criteria

1. Urine volume <500 mL/d
2. Urine sodium level <10 mEq/L
3. Urine osmolality > plasma osmolality
4. Urine red blood cells <50 per high-power field
5. Serum sodium concentration <130 mEq/L

[a]Only major criteria are necessary for the diagnosis of hepatorenal syndrome.

Other Renal Abnormalities in Cirrhosis

Patients with cirrhosis may also develop nonfunctional abnormalities of renal

function, particularly glomerular diseases, ATN, renal tubular acidosis (RTA), and drug-induced renal diseases.

The development of glomerular diseases occurs mainly in association with hepatitis B and C viruses as well as with alcoholic liver disease. Although there are no studies specifically assessing the incidence of ATN in cirrhosis, it may occur in patients who develop volume depletion in different clinical settings, such as septic or hypovolemic shock or after the administration of nephrotoxic drugs (42,44,45). If renal failure is secondary to volume depletion, renal function may improve after volume repletion and treatment of the precipitating factor. While hypovolemic shock related to gastrointestinal bleeding is easily recognized, the presence of septic shock may be more difficult to diagnose because of the subtle symptoms of bacterial infections in some patients with cirrhosis. Patients with cirrhosis are also at high risk of developing renal failure due to ATN when treated with aminoglycosides (44,45). Because of this high risk of nephrotoxicity and the existence of other effective antibiotics (i.e., third-generation cephalosporins) treatment with aminoglycosides should be avoided in patients with chronic liver disease. RTA may occur in cirrhosis of different etiologies, particularly primary biliary cirrhosis, autoimmune hepatitis, and alcoholic cirrhosis (46). The most common type of RTA in cirrhosis is the incomplete distal RTA. This form is usually subclinical and can be diagnosed only by measuring urinary pH following acid loading.

One of the most common types of renal failure physicians encounter when treating patients with cirrhosis is drug-induced renal dysfunction. Patients with cirrhosis may develop sodium and water retention or renal failure when treated with a variety of drugs, especially NSAIDs, aminoglycosides, diuretics, or vasodilators (45,47,48). Of these, the most important and commonly used in patients with cirrhosis are NSAIDS and diuretics. NSAIDs inhibit the enzymes cyclo-oxygenase 1 (COX-1) and COX-2 that are responsible for PG synthesis. PGs are important renal vasodilators that contribute significantly to maintaining normal renal perfusion. Renal failure has been reported to occur after a short treatment with a variety of NSAIDs, including indomethacin, aspirin, ibuprofen, naproxen, and sulindac (47,48,49,50,51). In these studies, the GFR quickly returned to the pretreatment values after cessation of the drug. However, it is not known whether renal failure may be reversible in patients treated for longer periods. Recent studies in patients with cirrhosis and ascites indicate that

COX-2 enzyme inhibitors do not cause renal failure when administered for a short period (51,52). The incidence of renal impairment during diuretic treatment ranges between 20% and 40% (48,53). This diuretic-induced renal failure is usually moderate and reversible after diuretic withdrawal and is related to an imbalance between the fluid loss from the intravascular space caused by diuretic treatment and the passage of fluid from the peritoneal compartment to the general circulation.

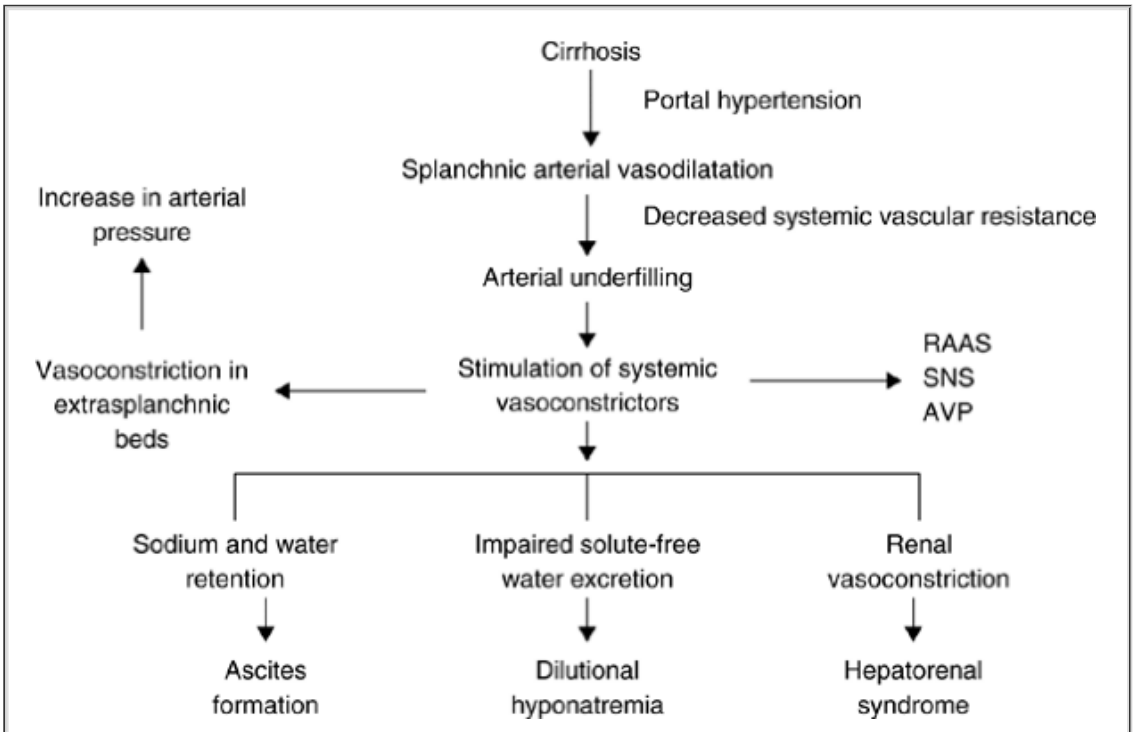
Factors Responsible for the Renal Functional Abnormalities in Cirrhosis

There are several intrarenal and extrarenal factors that have been proposed as potential mediators of functional renal abnormalities in cirrhosis. However, a common pathway for these derangements seems to be the development of an intense splanchnic arterial vasodilatation, which triggers an important compensatory response accounting for the three main renal function

abnormalities described earlier (Fig. 17.5). A concise review of these systems is provided in the subsequent text.

Extrarenal Factors

Most patients with cirrhosis and ascites show an overactivity of the two major vasoconstrictor and antinatriuretic systems, the RAAS and SNS (12,13,31,33). In addition, as described in the preceding text, there is also a nonosmotic hypersecretion of AVP. The activation of these systems is significantly more pronounced, once the patients develop HRS. These systems are activated in response to a reduced effective arterial blood volume, which is due to marked splanchnic vasodilatation. A large body of evidence indicates that the activation of these systems plays a major role in the increased tubular reabsorption of sodium and water in cirrhosis (12,13,31,33). However, in approximately 20% to 30% of patients with cirrhosis and ascites the serum levels of renin, aldosterone, and norepinephrine are normal. This is an unanswered question, but it has been suggested that increased renal tubular sensitivity to aldosterone or catecholamines (15,54), the decreased synthesis of a putative hepatic natriuretic factor (55), or existence of a "hepatorenal reflex" (see preceding text) could explain this phenomenon (33,34,35,54).



▪ **Figure 17.5** The pathogenesis of functional renal abnormalities in cirrhosis according to the arterial vasodilatation theory. RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; AVP, arginine vasopressin.

The activation of the RAAS and SNS is very intense in patients with HRS, which suggests that they participate in the pathogenesis of vasoconstriction of the renal circulation (12,13). Increased plasma levels of AVP enhance the solute-free water reabsorption in the collecting ducts and contribute to the water retention and

spontaneous dilutional hyponatremia (21). The pharmacologic interruption or blockade of the effectors of these three major vasoconstrictor systems (angiotensin II, norepinephrine, and AVP) in human or experimental cirrhosis results in marked reduction of total systemic vascular resistance and arterial hypotension, which suggests that these systems are activated as a homeostatic response to maintain arterial pressure within normal limits (56,57). The circulating levels of endothelin-1, an endothelial-derived peptide

with potent vasoconstrictor effect, are also increased in cirrhosis (58,59). These increased levels are probably due to an enhanced release of the peptide from the hepatic and/or splanchnic circulation (60,61). It has been proposed that the increased circulating levels and/or enhanced intrarenal production of endothelin may induce vasoconstriction of the renal circulation and play a role in the pathogenesis of HRS (62,63).

The plasma concentration of major natriuretic hormones, namely, atrial natriuretic peptide and brain natriuretic peptide, is increased in patients with cirrhosis and ascites (64,65,66). These increased plasma levels are due to an enhanced cardiac release and not due to a reduced systemic or hepatic clearance. Because natriuretic peptides have powerful effects on renal function (mainly vasodilator and natriuretic effects) and inhibit renin release, it is possible that increased natriuretic peptide levels may act as a homeostatic mechanism to counteract the effects of antinatriuretic and vasoconstrictor systems in the renal circulation.

Intrarenal Factors

In addition to extrarenal factors, a number of intrarenal factors with powerful effects on the renal circulation and/or tubular transport of sodium and water play an important role in the functional renal abnormalities of cirrhosis. Metabolites of arachidonic acid are the most widely studied metabolites of the intrarenal system. Arachidonic acid is metabolized in the kidney through three separate pathways involving different enzymes, namely, cyclo-oxygenases, lipoxygenases, and microsomal cytochrome P-450. The most important of these three pathways is dependent on cyclo-oxygenases and gives rise to PGs. The renal PGs, namely PGI_2 and PGE_2, by exerting vasodilatation have a protective effect on sodium and water transport and on renal circulation in conditions such as cirrhosis with ascites characterized by a marked activation of vasoconstrictor systems (17,47,67,68). Patients with cirrhosis and ascites without renal failure show increased renal production of vasodilator PGs compared with healthy subjects or patients without ascites (47,67,68). This increased production contributes to the maintenance of renal hemodynamics. This is demonstrated by the fact that PG synthesis inhibition by NSAIDs causes renal failure, particularly in patients with marked activation of vasoconstrictor systems (47,48,49,50,51). By contrast, patients with cirrhosis and HRS have a reduced renal production of PGs: PGE_2 and $PGF_{1\alpha}$ (1,68). These findings have been interpreted as indicative that the regulation of renal circulation in cirrhosis with ascites depends on an adequate balance between vasoconstrictor systems and intrarenal production of PGs. An imbalance between these vasoconstrictor systems and PGs likely contributes to renal vasoconstriction. However, eicosanoids with vasoconstrictor effects, such as cysteinyl leukotrienes or F_2-isoprostanes, may also participate in renal vasoconstriction in cirrhosis (67,68,69). In summary, the regulation of renal circulation in cirrhosis with ascites is a complex process which depends on an adequate balance between vasoconstrictor systems and intrarenal production of

PGs.

Nitric oxide (NO) locally synthesized in different structures within the kidney also participates in the regulation of renal function. Under normal circumstances, NO plays a role in the regulation of glomerular microcirculation by modulating the arteriolar tone and the contractility of mesangial cells. Moreover, NO facilitates natriuresis in response to changes in renal perfusion pressure, and regulates renin release (70). There is limited information on the role of NO in the regulation of renal function in cirrhosis, but it appears likely that NO interacts with PGs to maintain renal hemodynamics in cirrhosis with ascites (71,72,73).

Pathogenesis of Renal Functional Abnormalities

Although several theories have been proposed to explain the renal function abnormalities present in cirrhosis, the arterial vasodilatation theory still seems to be the most rational explanation for these derangements (31) (Fig. 17.5). This theory proposes that ascites formation and renal function abnormalities are the result of the effect of vasoconstrictor systems (RAAS and SNS) on the renal circulation activated as a homeostatic mechanism to improve the underfilling of the arterial circulation. This mechanism is qualitatively similar to what occurs in states of volume depletion. However, in volume depletion the activated mechanisms responsible for sodium retention are turned off as soon as the extracellular fluid volume reaches normal values, thereby preventing the appearance of edema. By contrast, in edematous conditions like cirrhosis, antinatriuretic mechanisms remain activated despite a progressive expansion of the extracellular fluid volume and edema formation.

According to the arterial vasodilatation theory, the initial abnormality causing renal sodium retention would be a sinusoidal portal hypertension leading to a marked arterial vasodilatation located mainly in the splanchnic circulation. Potential substances involved in this vasodilatation include NO and vasodilator peptides. NO seems to be the most important substance mediating vasodilatation in this territory (74). Splanchnic arterial vasodilatation would result in an abnormal distribution of blood volume with reduction of effective

arterial blood volume (i.e., the blood volume in the heart, lungs, and central arterial tree that is sensed by arterial receptors) and subsequent renal sodium and water retention due to the activation of vasoconstrictor and antinatriuretic factors. In the preascitic stage (increased total blood volume without ascites or edema), the retained fluid would suppress sodium and water retention and reset fluid balance at an upper level of blood volume. In addition, there is a homeostatic increase in cardiac output as a result of a decreased afterload. However, as the disease progresses more and more vasodilatation would be produced in the splanchnic circulation and the effective arterial blood volume would no longer be maintained by the increased total blood volume, and leakage of fluid from the splanchnic circulation to the peritoneal cavity is what results as ascites. At this point, a persistent activation of vasoconstrictor and antinatriuretic systems occurs in an attempt to maintain a normal effective arterial blood volume and arterial pressure. Vasodilatation is so intense that the increased cardiac output is not sufficient to maintain circulatory homestasis. The continuous leakage of intravascular fluid to the peritoneal cavity likely explains the paradox of unrelenting activation of antinatriuretic systems in the setting of an increased extracellular fluid volume. In very advanced stages of the disease, the disturbance in the splanchnic circulation is so intense that systemic

hemodynamics can be maintained only at the expense of severe vasoconstriction in most vascular beds, including the renal circulation, and HRS develops. Recent studies suggest that the development of HRS occurs in the setting of a reduction in cardiac output, indicating that the progression of circulatory and renal dysfunction in cirrhosis are not only due to splanchnic vasodilatation but also due to a reduction in cardiac output (30,75). This suggests that HRS may be the consequence of a fall in cardiac output in the setting of marked splanchnic vasodilatation (30).

Management of the Functional Renal Abnormalities in Cirrhosis

Sodium Retention and Ascites

The management of patients with cirrhosis and ascites is discussed in detail in Chapter 19.

Solute-Free Water Retention and Hyponatremia

Although hyponatremia is associated with a poor prognosis, there are no studies indicating that correcting hyponatremia per se is associated with an improved survival. Treating the underlying liver disease is the most important aspect of treatment and therefore evaluation for liver transplantation is mandated in all patients presenting with spontaneous dilutional hyponatremia. At present, no approved pharmacologic therapy exists for dilutional hyponatremia and the only therapeutic measure that may slow the progressive decrease in serum sodium concentration is water restriction to 1 L/day. Fluid restriction in patients with cirrhosis and dilutional hyponatremia is not very effective in raising serum sodium levels. However, in a randomized placebo-controlled study comparing placebo versus a V2 receptor of AVP antagonist, fluid restriction did not increase serum sodium, but it did prevent it from lowering further (76). The administration of hypertonic saline solutions is not recommended because it invariably leads to further expansion of extracellular fluid volume and marked accumulation of ascites and edema (19). Moreover, these patients also have marked sodium retention and therefore their sodium intake must also be restricted. Also, as mentioned earlier, certain circumstances such as the use of NSAIDs and large-volume paracentesis without volume expansion with albumin should be avoided.

Pharmacologic approaches to the management of dilutional hyponatremia in cirrhosis have focused on inhibiting the actions of AVP. The most promising types of drugs are the antagonists of the V2 receptor of AVP which are under clinical investigation (19,76,77,78). These agents selectively antagonize the water-retaining effect of AVP in the cortical collecting duct and increase solute-free water excretion, decrease body weight, and raise serum sodium levels in patients with cirrhosis, ascites, and dilutional hyponatremia (76,78). Results of further large-scale phase III trials will certainly help define the usefulness of V2 receptor antagonists in clinical practice.

Renal Vasoconstriction and Hepatorenal Syndrome

Prevention

HRS can be prevented in two clinical settings. In patients with SBP, the administration of albumin (1.5 g/kg body weight at diagnosis of infection and 1

g/kg body weight 48 hours later) prevents the circulatory dysfunction and subsequent development of HRS (39). Because SBP may trigger HRS by decreasing effective arterial blood volume, the rationale for albumin administration is to prevent arterial underfilling and subsequent activation of vasoconstrictor systems during the infection (39). The incidence of HRS in patients with SBP receiving albumin together with antibiotic therapy is 10%, compared to that of 33% in patients not receiving albumin. Most importantly, hospital mortality was lower in patients receiving albumin (10%) versus those not receiving plasma expansion (29%) (36). In patients

with acute alcoholic hepatitis, the administration of pentoxifylline, an inhibitor of tumor necrosis factor-α (400 mg t.i.d. orally for 28 days) reduces the incidence of HRS and mortality (8% and 24%, respectively) with respect to a control group (35% and 46%, respectively) (36). Although there are no follow-up studies confirming these results, these two approaches are widely used in the clinical setting owing to the wide accessibility of albumin and pentoxifylline in most centers.

Pharmacologic therapy

While considering pharmacologic therapy, all patients with HRS should be evaluated for liver transplantation because it is the best treatment as it removes the diseased liver that causes disturbances in renal function. A variety of pharmacologic interventions have been attempted over the years to treat HRS. Drugs with renal vasodilator activity were used in patients with HRS in an attempt to counteract the effect of vasoconstrictor factors on renal circulation. Dopamine was the first drug used because of its renal vasodilatory effect when given in suppressor doses. Studies specifically assessing the effects of dopamine on renal function in series of patients with HRS showed no or only minor effects on GFR; therefore, there are no data to support its use in clinical practice in patients with HRS (79,80). The second type of renal vasodilators used in patients with HRS are PGs and PG analogs. The rationale for the use of PGs is based on the fact that renal vasoconstriction in HRS is partly related to a reduced intrarenal synthesis of vasodilator PGs. Unfortunately, no consistent beneficial effects on renal function were observed after the intravenous or intra-arterial administration of PGA_1 or PGE_2 (1,15). The oral administration of misoprostol (a PGE_1 analog) was found to improve renal function in one study but this beneficial effect was not confirmed in a subsequent investigation (81,82). The problem with this drug is the high incidence of side effects, especially diarrhea. Other approaches such as the use of endothelin blockers (BQ123) and *N*-acetylcysteine seem to be promising, but larger uncontrolled as well as controlled studies are needed to confirm their role in the therapy of HRS (83,84).

Systemic vasoconstrictors with plasma expansion seem to be the best therapy because several studies have confirmed their beneficial role in HRS, particularly type 1 HRS (80,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101) Although using vasoconstrictors in a condition characterized by renal vasoconstriction seems paradoxical, the rationale is based on the fact that the initial event in the pathogenesis of HRS is an arterial splanchnic vasodilatation causing activation of endogenous vasoconstrictors. Vasoconstrictors used for treatment of HRS include vasopressin analogs (terlipressin) that have a potent vasoconstrictor effect through the action on V1 receptors with a low V2 receptor agonist activity, somatostatin analogs (octreotide) that act as glucagon inhibitors, and α-adrenergic agonists (midodrine and noradrenaline) that also

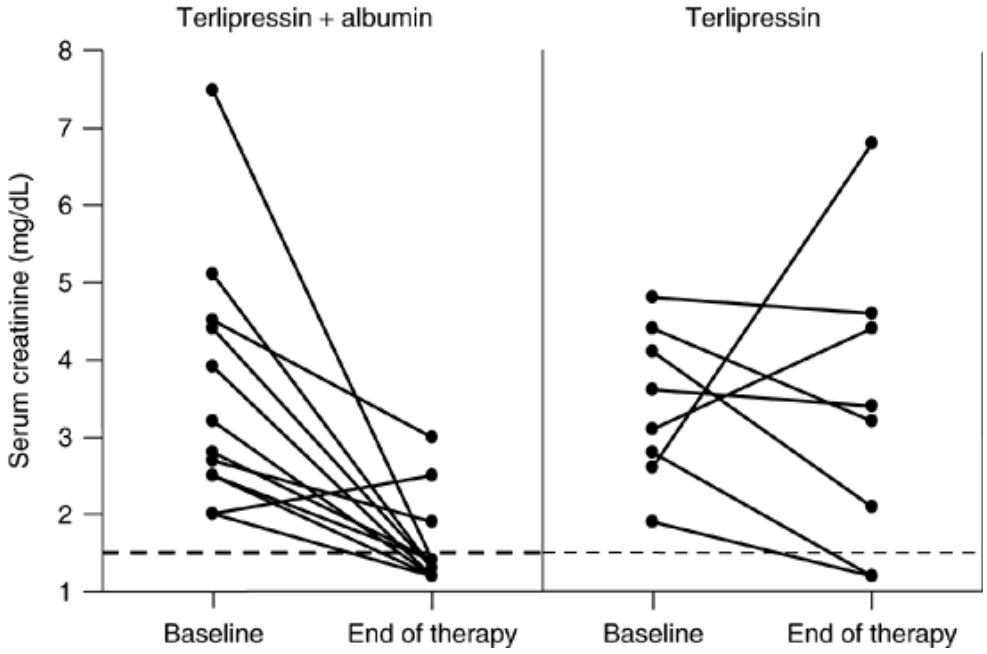
cause vasoconstriction by acting on α_1-adrenergic receptors. In most studies, vasoconstrictors have been given in combination with plasma expansion (albumin), which seems to improve the efficacy of treatment.

Vasopressin analogs have a marked vasoconstrictor effect in the splanchnic circulation and have been used for many years in the management of acute variceal bleeding in patients with cirrhosis. The administration of terlipressin and albumin is associated with a significant improvement in GFR and reduction of serum creatinine level below 1.5 mg/dL in 42% to 92% of patients with type 1 HRS (89,90,91,92,93,94,95,96,97,98,99,102) (Fig. 17.6). Ornipressin and vasopressin, although effective in HRS, caused significant ischemic side effects in approximately 30% to 40% of patients and were abandoned (85,86,87). The incidence of ischemic side effects with terlipressin is lower and occurs only in 5% to 10% of cases (89,90,91,92,93,94,95,96,97,98,99,102). Patients with Child-Pugh scores of 13 or higher and those who do not receive albumin expansion do not seem to respond well to this treatment (90,91). There may be recurrence after stopping treatment and a repeat course of terlipressin with albumin is usually effective (90,91). In addition, survival of responders to terlipressin is better than that of nonresponders (90,91).

Administration of midodrine in association with octreotide, an inhibitor of the release of glucagon and other vasodilator peptides, and albumin also improves renal function in patients with cirrhosis and HRS, although information about this therapeutic approach is limited to only two studies with a total of less than 20 patients (80,100). In all cases there was a marked improvement in renal perfusion and GFR, and a suppression of renin, aldosterone, norepinephrine, and AVP to normal or near normal levels. Interestingly, octreotide is ineffective when administered alone (103). These results require confirmation in a larger group of patients. Finally, the administration of noradrenaline (0.5 to 3 mg/h) for a minimum of 5 days in association with intravenous albumin resulted in a significant improvement of renal function in a small group of 12 patients with cirrhosis and type 1 HRS (101). Reversal of HRS was observed in 10 patients in association with an increase in mean arterial pressure and a marked reduction in renin and aldosterone. There was an episode of reversible myocardial hypokinesia, and no other ischemic side effects (101).

The information about the use of vasoconstrictors in patients with HRS is somewhat limited because there are no large-scale placebo controlled studies showing that they are superior to no treatment. However, on the basis of published studies, some recommendations can

be made while awaiting the results of large randomized controlled trials:



▪ **Figure 17.6** The effects of terlipressin and albumin compared to terlipressin alone on serum creatinine levels in patients with type 1 hepatorenal syndrome. (Reprinted with permission from Ortega R, Ginès P, Uriz J, et al. Terlipressin therapy with and without albumin for patients with hepatorenal syndrome. Results of a prospective, non-randomized study. *Hepatology* 2002;36:941–948.)

1. These drugs should be used for at least 7 to 20 days, because the improvement of renal function usually occurs slowly.
2. Therapy should be aimed at reducing serum creatinine level below 1.5 mg/dL (130 µmol/L).
3. The following are the recommended dosages:
    A. Terlipressin: 0.5 mg intravenously every 4 hours; can increase dose in a stepwise manner (i.e., every 2 days) to 1 mg/4 hours and then up to 2 mg/4 hours in cases where there is no reduction of serum creatinine level.
    B. Midodrine: 7.5 mg orally three times daily with an increase to 12.5 mg three times daily if needed and octreotide: 100 µg subcutaneously three times daily with an increase to 200 µg three times daily, if needed.
    C. Noradrenaline : Titration of 0.5 to 3 mg/hour continuous intravenous infusion.
4. The concomitant administration of albumin (1 g/kg on the first day, followed by 20 to 40 g/day) as a plasma expander is recommended in all cases, although its beneficial effect has not been tested in randomized studies.
5. Contraindications: Patients with coronary artery disease, peripheral vascular

disease, and/or cerebrovascular disease due to the potential risk of ischemic events.

6. Because of limited information and the possibility of side effects, treatment with vasoconstrictors should probably be restricted at present to patients with type 1 HRS.

The most important goal of pharmacologic therapy in HRS is that of successfully reversing renal failure so that potential liver transplantation candidates can undergo transplantation with less morbidity and have survival similar to patients without HRS. Patients treated successfully with terlipressin and albumin before liver transplantation have a good posttransplantation outcome and a survival similar to that of patients without HRS, who underwent transplantation (104).

Transjugular intrahepatic portosystemic shunt

Transjugular intrahepatic portosystemic shunt (TIPS) is a nonsurgical method of portal decompression that has evolved as an alternative therapy for patients with cirrhosis who are bleeding from esophageal or gastric varices and are refractory to endoscopic and medical treatment. Although the use of TIPS has been evaluated for the treatment of refractory ascites, there are very scarce data regarding its use in the management of HRS (100,105,106,107,108). The rationale for using TIPS is based on the fact that reducing portal pressure may improve circulatory function and suppress RAAS and SNS activities. Uncontrolled studies indicate that TIPS may improve renal function and GFR as well as reduce the activities of RAAS and SNS in patients with cirrhosis and type 1 HRS (100,106,107,108). The effects on renal function and the clinical course of patients after TIPS insertion are variable. Improvement in renal function after TIPS placement alone is generally slow with success in approximately 60% of patients (100,105,106,107,108). Studies assessing TIPS for type 1 HRS have included patients with relatively preserved

liver function. Patients with a Child-Pugh score of 12 or more were excluded because of the risk of worsening liver failure and/or hepatic encephalopathy. Unfortunately, most patients with type 1 HRS fall in this category. An approach combining the use of vasoconstrictors with TIPS for treatment of type 1 HRS was reported showing that patients with preserved liver function (Child-Pugh score <12) who responded to midodrine, octreotide, and albumin and then had a TIPS inserted had an excellent outcome with renal function that continued to improve, and completely normalized (100). However, larger studies are needed to confirm these results.

In patients with type 2 HRS, TIPS improves renal function and reduces ascites (107,108,109,110). However, experience from a large series of patients with cirrhosis undergoing TIPS for refractory ascites indicates that those with a Child-Pugh score of 12 or more, hepatic encephalopathy and severe coagulopathy have a worse outcome if TIPS is placed (109,110). Although uncontrolled studies suggest that TIPS alone improves prognosis in patients with type 1 and 2 HRS (108), the impact of this therapy on patient survival remains to be assessed.

Dialysis

Hemodialysis and peritoneal dialysis have been used in the management of patients with HRS, and sporadic cases of improvement of renal function have

been reported. Unfortunately, there are no controlled studies evaluating the effectiveness of dialysis in HRS. Uncontrolled studies suggest that it is hardly effective because most patients die during treatment and there is a high incidence of severe side effects, including arterial hypotension, coagulopathy, and gastrointestinal bleeding. Although hemodialysis is not routinely recommended in HRS, it may be a reasonable option in suitable liver transplantation candidates as a bridge to transplantation when there is no response to vasoconstrictors or TIPS, or in patients who develop severe volume overload, metabolic acidosis, or refractory hyperkalemia (42). Continuous arteriovenous or venovenous hemofiltration have also been used but their efficacy also remains to be determined. Data on the extracorporeal albumin dialysis system, that is molecular adsorbent recirculating system (MARS) seem to be beneficial, but only one study is available. In this study of only 13 patients with Child-Pugh C cirrhosis and type 1 HRS, the authors reported a significant decrease in bilirubin and creatinine levels; an improvement in serum sodium level, urine volume, and mean arterial blood pressure; and decreased mortality (111). Additionally, a study using Prometheus, another extracorporeal liver support system that combines removal of albumin-bound substances and water-soluble substances, was reported to be safe and beneficial in a small group of patients with type 1 HRS (112) However, these results require further evaluation to consider albumin dialysis as a therapy for HRS.

Liver transplantation

Liver transplantation is the best treatment for patients with HRS. This therapy offers a cure to both the diseased liver and the circulatory and renal dysfunction. The long-term outcome of patients with cirrhosis and HRS treated by liver transplantation is good (survival is 85% at 1 year and 73% at 3 years), although the presence of HRS is associated with increased morbidity and early mortality after transplantation (113). Unfortunately, transplantation for type 1 HRS is limited by the fact that a significant proportion of patients die before the surgery because they have a short survival and a prolonged waiting time in most centers (114). Patients with type 1 HRS have a very poor prognosis and this group of patients should be given immediate priority (43,114). Additionally, patients with type 2 HRS and a MELD score of 20 and more also have a poor outcome and should be given high priority for transplantation (43). If patients are successfully treated with pharmacologic therapy, the outcome appears to be similar to that of patients without HRS (104,114).

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Chapter 18
Pulmonary Manifestations of Liver Disease

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Key Concepts

- Liver disease, most commonly cirrhosis, is associated with unique pulmonary vascular abnormalities independent of intrinsic cardiopulmonary disease.
- Ten percent to 20% of patients with cirrhosis develop hepatopulmonary syndrome (HPS), which results when vasodilatation in the pulmonary microvasculature leads to hypoxemia. The presence of HPS increases mortality in cirrhosis. Currently, liver transplantation is the only established therapy for HPS. However, when HPS is severe the outcome of liver transplantation is adversely affected.
- Portopulmonary hypertension (POPH) results when vascular remodeling increases pulmonary vascular resistance and elevates mean pulmonary artery pressure. POPH occurs in 4% to 8% of patients with cirrhosis and, when moderate or severe, it prohibitively increases mortality after liver transplantation. Medical therapies can improve pressures, but no studies clearly support that liver transplantation improves POPH.
- Screening for HPS and POPH is important in cirrhosis because the presence of these disorders influence survival and liver transplantation candidacy.

The presence of dyspnea can be elicited in as many as 70% of patients with cirrhosis undergoing evaluation for liver transplantation (1). In addition, abnormalities in pulmonary function and impaired gas exchange may occur in as many as 45% to 50% of patients (2). The most common causes of these abnormalities and of respiratory symptoms in general are disorders that occur in patients independent of the presence or absence of chronic liver disease (i.e., chronic obstructive pulmonary disease and interstitial lung disease). Additionally, symptoms may result from patient deconditioning, muscular wasting, or the presence of tense ascites/hepatic hydrothorax (see Chapter 19). Finally, certain liver diseases may be associated with specific pulmonary abnormalities, such as panacinar emphysema in α_1-antitrypsin deficiency (Chapter 37) and fibrosing alveolitis and granulomas in primary biliary cirrhosis (Chapter 24).

In a subset of patients, however, two distinct disorders, the hepatopulmonary syndrome (HPS) and portopulmonary hypertension (POPH), are observed. This chapter reviews the epidemiology, clinical characteristics, and treatment of these

pulmonary vascular complications of liver disease.

Hepatopulmonary Syndrome

Definition

HPS is commonly defined as a widened age-corrected alveolar–arterial oxygen gradient of room air with or without hypoxemia that occurs as a result of intrapulmonary vasodilatation in the presence of hepatic dysfunction or portal hypertension (3,4). Fluckiger first described the association between pulmonary dysfunction and liver disease over 100 years ago (5), but the term *hepatopulmonary syndrome* was not used until 1977 (6) when the role of intrapulmonary vasodilatation as the major contributor to gas exchange abnormalities was recognized.

Epidemiology and Disease Associations

Although detectable intrapulmonary vasodilatation may be present in 40% of patients with cirrhosis (7), only 8% to 15% will develop impaired oxygenation, leading to significant functional limitations (3). Prior definitions of HPS emphasized the need to exclude intrinsic cardiopulmonary disease to establish the diagnosis (4). However, recent data supports the observation that HPS may occur in the setting of other cardiopulmonary abnormalities (8,9).

Typically, HPS is diagnosed in subjects with cirrhosis and portal hypertension regardless of the underlying etiology of liver disease. Whether the prevalence or severity of HPS correlates with the degree of hepatic synthetic dysfunction and the severity of portal hypertension remains unclear (7,8,10,11,12,13). Cases of HPS have been described in patients with portal hypertension without cirrhosis (e.g., prehepatic portal hypertension, nodular regenerative hyperplasia, congenital hepatic fibrosis, and hepatic venous outflow obstruction) (14,15,16,17) and in patients with hepatic dysfunction in the absence of established portal hypertension (e.g., acute and chronic hepatitis) (18,19). A syndrome similar to HPS has been described in pediatric congenital cardiovascular abnormalities associated with altered hepatic venous drainage to the lungs (20,21) and in a case of metastatic carcinoid tumor (22).

Pathogenesis

The most important alteration in HPS is the dilatation of the precapillary and postcapillary pulmonary vasculature (23), which leads to impaired oxygenation of venous blood as it passes through the lung (24,25).

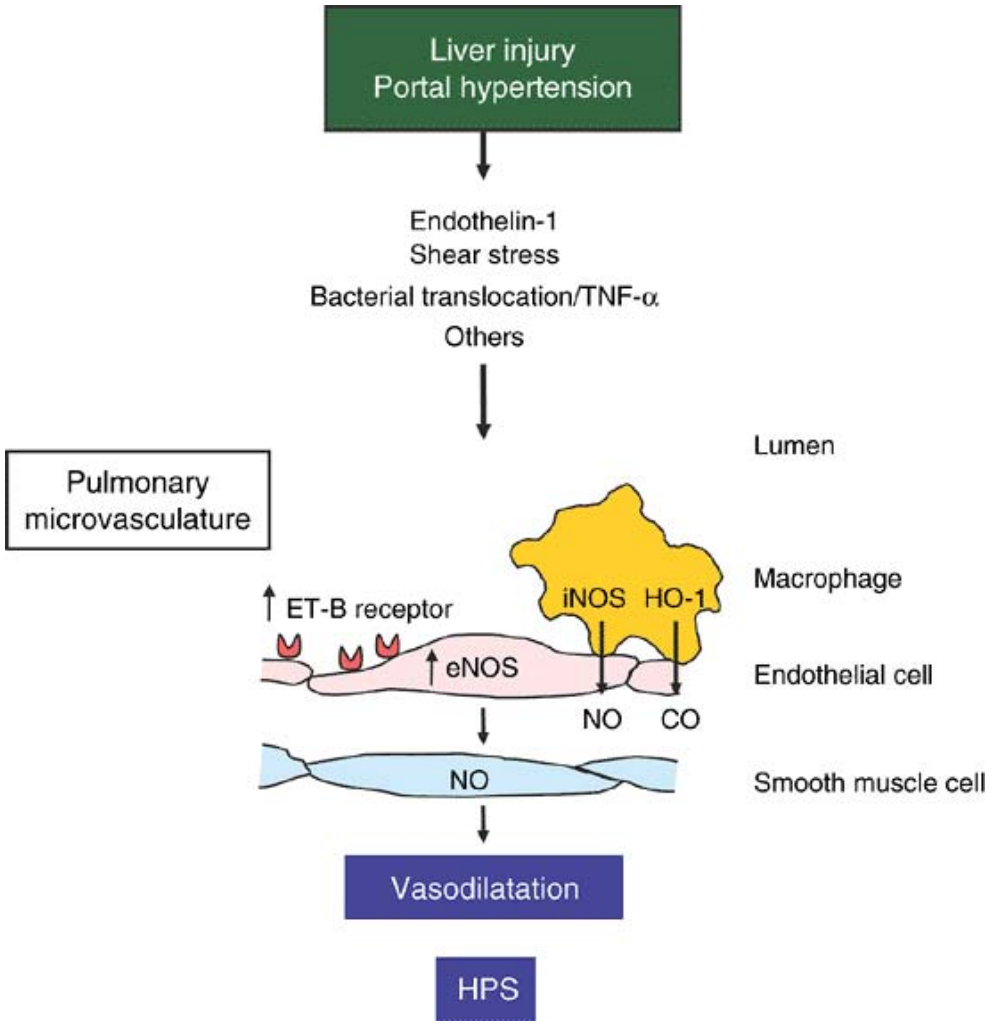
Human studies have demonstrated increased pulmonary production of nitric oxide (NO). Exhaled NO levels are increased in patients with cirrhotic HPS and normalize after liver transplantation (26,27,28) as HPS resolves. In addition, inhibition of the production or action of NO with N^G-nitro-L-arginine methyl ester (L-NAME) or methylene blue, respectively, transiently alleviates HPS (29,30,31). However, the mechanisms of increased NO production in human HPS and its relationship to the degree of liver injury and portal hypertension and/or the hyperdynamic circulation observed in cirrhosis remain unclear. In addition, it is not known whether other vasoactive mediators such as carbon monoxide (CO), the level of which has recently been found to be increased in human HPS, contribute to vasodilatation (32).

Common bile duct ligation (CBDL) in rats (33,34) has been used as an experimental model of HPS because other animal models of cirrhosis and/or portal hypertension (i.e., thioacetamide-induced cirrhosis or partial portal vein ligation) do not result in the development of HPS (35). In this model, increased pulmonary production of NO appears to be a central event in the development of HPS through increased pulmonary vascular expression and activity of endothelial nitric oxide synthase (eNOS) (36,37,38,39). Enhanced hepatic production and release of endothelin-1 (ET-1) and shear stress–mediated increase in pulmonary endothelial endothelin B receptor (ET-B) expression appear to be major contributors to the observed increase in eNOS expression and activity (40,41,42). These events lead to enhanced activation of eNOS by ET-1 through the ET-B receptor and are followed by the accumulation of intravascular macrophages and the production of inducible nitric oxide synthase (iNOS) (36,38,39) and heme oxygenase-1 (HO-1) (38,43), leading to increased NO and CO production, respectively, which result in vasodilatation. Increased tumor necrosis factor-α (TNF-α) production and ET-1 itself appear to contribute to endothelial alterations and macrophage accumulation and activation (42,44) as in CBDL animals. TNF-α inhibition through intestinal decontamination or administration of pentoxifylline and selective ET-B receptor antagonists ameliorate HPS (42,44,45). Figure 18.1 summarizes the current understanding of mechanisms of pulmonary microvascular dilatation in experimental HPS. However, whether similar mechanisms are operative in human disease is unknown.

Clinical Features

The diagnosis of HPS may be overlooked or delayed because many patients with HPS are asymptomatic or their respiratory symptoms may be attributed to intrinsic lung disease; therefore, a high index of suspicion is necessary to establish the diagnosis. In symptomatic patients, the insidious onset of dyspnea (1) is the most frequent complaint. Platypnea (increased dyspnea upon standing) is a "classical" complaint in HPS and is attributed to the predominance of vasodilatation in the lung bases and the increased "shunting" through these regions in the upright position, leading to hypoxemia (46). However, the prevalence, sensitivity, and specificity of platypnea remain undefined. Spider angiomata, digital clubbing, and cyanosis are also commonly described in subjects with HPS but have not been prospectively evaluated as diagnostic indicators. Chest x-rays are most commonly normal but may reveal lower lobe interstitial changes that may be confused with interstitial lung disease (47). Pulmonary function tests typically demonstrate well-preserved spirometry and lung volumes; however, the diffusing capacity of lung for carbon monoxide (DLCO) is often significantly reduced. However, the DLCO is also commonly decreased in cirrhosis in the absence of

HPS, and the diagnostic utility of a reduced value has not been established (48).



▪ **Figure 18.1** Proposed mechanisms of hepatopulmonary syndrome (HPS) based on findings in experimental models. Liver injury and/or portal hypertension trigger the production of cytokines and vasoactive mediators that increase vascular shear stress. Pulmonary microvascular dilatation is initiated by hepatic endothelin-1 production and release and endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) production through an increased number of endothelial endothelin B receptors. Macrophages that accumulate in the microvasculature also contribute to vasodilatation by producing NO from inducible nitric oxide synthase (iNOS) and carbon monoxide (CO) from heme oxygenase-1 (HO-1).

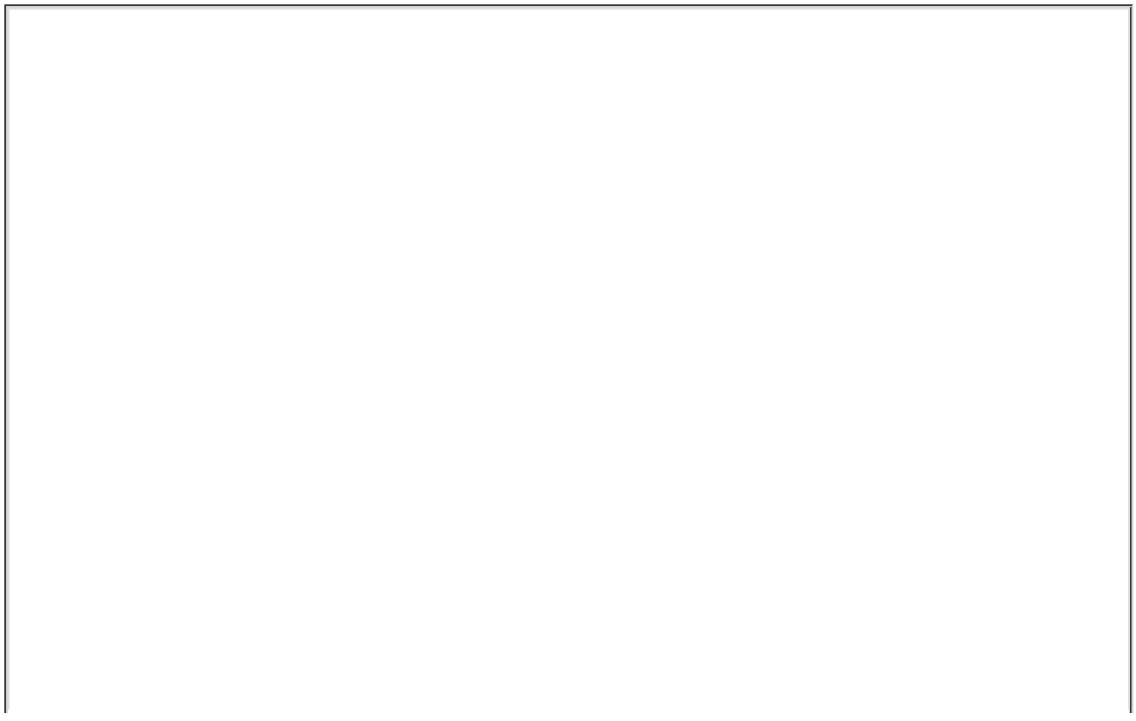
Evaluation for Hepatopulmonary Syndrome

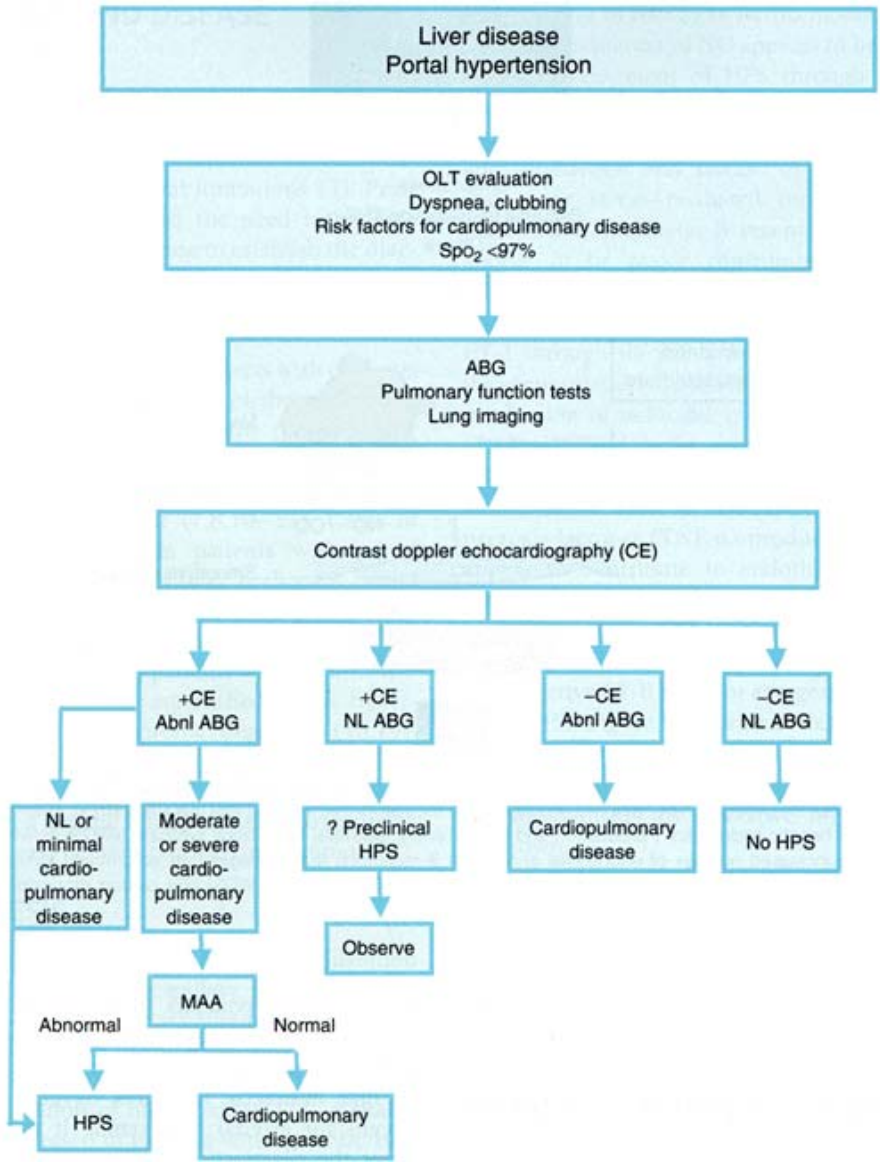
Pursuing the diagnosis of HPS is appropriate in patients complaining of dyspnea and/or in those who display digital clubbing or cyanosis. In patients being considered for liver transplantation, regardless of the presence of symptoms, screening is important because the presence of HPS may influence transplant candidacy and priority. Figure 18.2 summarizes one approach to the diagnosis of HPS.

The diagnosis of HPS is established by (i) the presence of arterial gas exchange

abnormalities and (ii) documenting intrapulmonary vasodilatation. Gas exchange abnormalities, detected by arterial blood gas measurements, are defined as a widened age-corrected alveolar–arterial oxygen gradient ([PAO_2 – PAO_2] >15 to 20 mm Hg) with or without hypoxemia (PaO_2 < 70 mm Hg) (4). Obtaining arterial blood gases in the sitting position may enhance the likelihood of detection of hypoxemia in HPS because of the predominance of vasodilatation in the lower lung fields. Pulse oximetry is a noninvasive screening modality that indirectly measures oxygen saturation and screens for arterial hypoxemia. It may be useful to target the diagnostic evaluation for HPS at patients who have a higher likelihood of advanced disease (PaO_2 <70 mm Hg) (49). However, the threshold oximetry value for detecting hypoxemia in cirrhosis is higher than that typically expected (≤97%), and it is not possible to detect less-advanced disease with oximetry alone (normal PaO_2, widened PAO_2 – PaO_2) (49,50). The clinical significance of these latter gas exchange abnormalities is not well defined (51). Contrast echocardiography, lung perfusion scanning, pulmonary angiography, and high-resolution chest computed tomography (CT) scanning are modalities available to detect intrapulmonary vasodilatation. Two-dimensional transthoracic contrast echocardiography is the most sensitive and most commonly employed screening technique. The contrast agent utilized is agitated saline that creates microbubbles visible on echocardiography. Visualization of intravenously

administered saline microbubbles in the left cardiac chambers after three cardiac cycles is considered a positive test (52,53) (Fig. 18.3). Normally, microbubbles should not be visualized in the left side of the heart, whereas immediate visualization of injected contrast indicates intracardiac shunting. Transesophageal contrast echocardiography may increase the sensitivity of detecting intrapulmonary vasodilatation compared to transthoracic echocardiography but the former is a more invasive and expensive modality (52,53). Echocardiography can also assess cardiac function and estimate pulmonary arterial systolic pressure and is useful for screening for cardiac dysfunction and pulmonary hypertension. As many as 40% of patients with cirrhosis and normal arterial blood gases may have a positive contrast echocardiogram, suggesting that mild intrapulmonary vasodilatation insufficient to alter gas exchange and cause HPS is common (7).



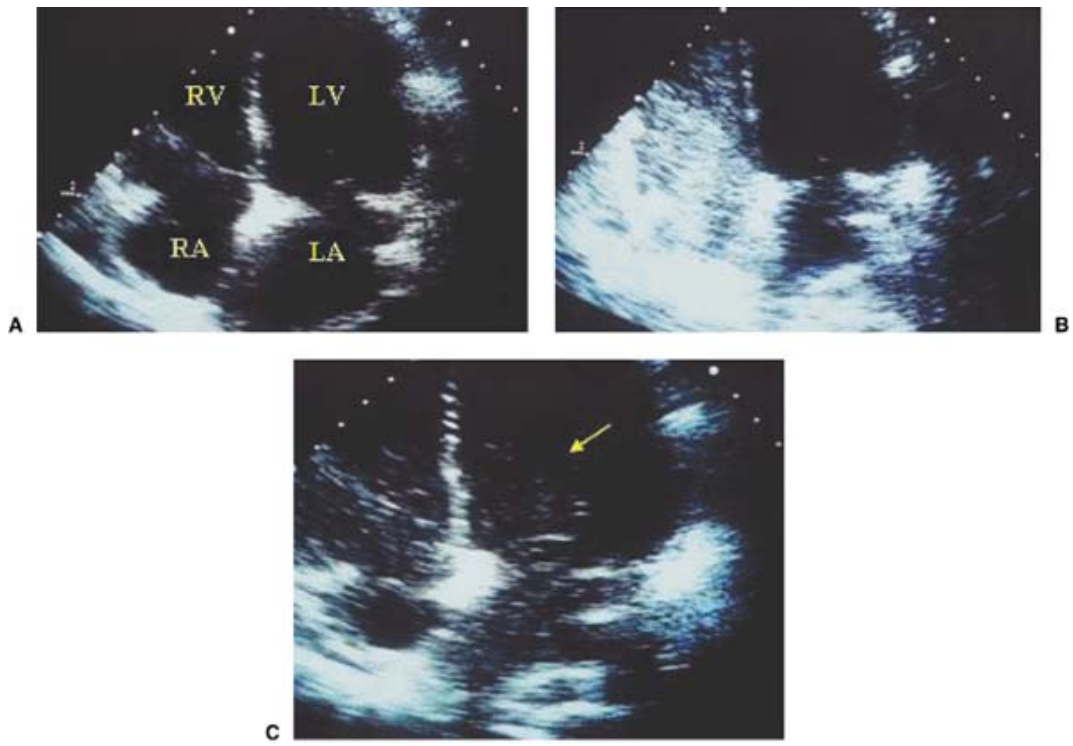


▪ **Figure 18.2** Diagnostic approach to hepatopulmonary syndrome (HPS). See text for details. OLT, orthotopic liver transplantation; Spo_2, pulse oximetry oxygen saturation; ABG, arterial blood gas; CE, contrast echocardiography; Abnl, abnormal; NL, normal; ^{99m}Tc MAA, technetium- 99m–labeled macroaggregated albumin scan.

Radionuclide lung perfusion scanning, using technetium-99m–labeled macroaggregated albumin particles (^{99m}Tc MAA scan), is another method for detecting intrapulmonary vasodilatation (Fig. 18.4). In this test, macroaggregated albumin particles 50 to 100 μm in size are injected intravenously. Normally, all particles are

trapped in the lung microvasculature. In HPS, some particles escape through dilated pulmonary capillaries and lodge in beds supplied by systemic arteries. Quantitative imaging of the lung and brain using a standardized methodology allows calculation of a shunt fraction (8). The ^{99m}Tc MAA scan offers one

significant advantage over contrast echocardiography; a positive scan (shunt fraction >6%) is specific for the presence of HPS even in the setting of coexistent intrinsic lung disease (8) and is valuable in determining the relative importance of intrapulmonary vasodilatation or the underlying pulmonary process as the major contributor for observed gas exchange abnormalities. However, as a screening test for intrapulmonary vasodilatation, ^{99m}Tc MAA scanning is less sensitive than contrast echocardiography.



▪ **Figure 18.3** Contrast echocardiogram for detecting intrapulmonary vasodilatation. **A:** Parasternal four-chamber view of the heart before the administration of agitated saline contrast. RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle. **B:** Four-chamber view immediately after the administration of agitated saline contrast into the antecubital vein, leading to the demonstration of the presence of echogenic microbubbles in the right atrium and ventricle. **C:** Visualization of echogenic microbubbles in the left atrium (*arrow*) and ventricle three cardiac cycles after visualization on the right due to intrapulmonary vasodilatation in a patient with hepatopulmonary syndrome. Intracardiac shunting results in immediate passage of microbubbles from the right to left chambers without a three-cycle delay and can be excluded using this technique.

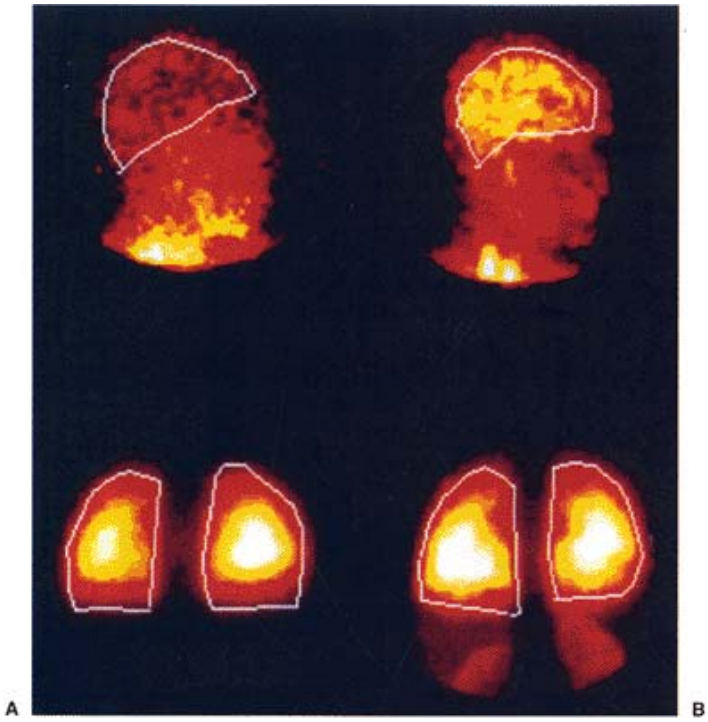
Two types of angiographic findings have been reported in HPS: Type 1—diffuse "spongiform" appearance of pulmonary vessels during the arterial phase—and type 2—small discrete arteriovenous communications. However, many patients with documented HPS have normal angiograms and the test is invasive. Therefore, pulmonary angiography is not a useful screening test.

High-resolution chest CT scan has been recently demonstrated to be a useful modality to detect dilated pulmonary vessels in HPS (54). The degree of

dilatation observed on CT scan can be correlated with the severity of gas exchange abnormalities in patients with HPS, suggesting that quantitation of intrapulmonary vasodilatation may be possible, but further studies are warranted to define the role of CT scan in assessing the presence and severity of HPS.

Prognosis and Natural History

The natural history of HPS is incompletely characterized, but available data indicates that most patients develop progressive intrapulmonary vasodilatation and worsening gas exchange (55) over time. Mortality is significant in patients with HPS (55) and may be due in part to causes directly related to intrapulmonary vasodilatation.



▪ **Figure 18.4** Technetium-99m–labeled macroaggregated albumin (^{99m}Tc MAA) scanning. **A:** A normal ^{99m}Tc MAA scan from a patient without hepatopulmonary syndrome (HPS) with regions of interest drawn around the lungs and cerebrum. In the absence of intrapulmonary vasodilatation, there is minimal passage of intravenously administered labeled albumin through the lungs and signal intensity is low in the cerebrum. Shunting is quantified by comparing the relative signal intensity in the lung and the brain. **B:** An ^{99m}Tc MAA scan in HPS demonstrates significant cerebral uptake because of passage of labeled albumin through the dilated pulmonary microvasculature. (Reproduced from Abrams GA, Nanda N, DubovskyE, et al. Use of macroaggregated albumin lung perfusion scan to diagnose hepatopulmonary syndrome: a new approach. *Gastroenterology* 1998;114:308 with permission.)

A recent prospective study has evaluated the natural history of HPS in a cohort of

111 patients with cirrhosis among whom 24% had HPS (10). The median survival among patients with HPS was significantly shorter (10.6 months) compared to those without HPS (40.8 months). Mortality remained higher in patients with HPS after adjusting for severity of underlying liver disease and after excluding patients who underwent liver transplantation during follow-up. The causes of death in patients with HPS were mainly due to complications of hepatocellular dysfunction and portal hypertension and correlated with the severity of hypoxemia in HPS. Analysis of data from an HPS registry has confirmed higher mortality in patients with HPS, although median survival in HPS was longer than that in the study mentioned earlier (56).

Therapy

A number of agents including somatostatin, almitrine, indomethacin, intravenous L-NAME, and plasma exchange have been tried unsuccessfully to treat HPS (15). Aspirin increased arterial oxygenation in two children with HPS (57), and a case report (58) and a subsequent open-label trial of 15 patients (59) reported that using garlic powder administered for a minimum of 6 months led to significant improvement in PaO_2 (>10 mm Hg) in 6 patients (40%). Infusion of methylene blue, which inhibits the effect of NO on soluble guanylate cyclase, was also found to transiently improved oxygenation in two reports comprising a total of eight patients (30,31). In addition, the inhibition of NO production with inhaled L-NAME also transiently improved oxygenation in one patient (29). Finally, a case report found that norfloxacin use contributed to improvement in oxygen saturation (60). None of these agents is clearly effective as medical therapy for HPS and randomized multicenter trials are needed. On the basis of our current understanding of experimental HPS, agents that modulate endothelin signaling, TNF-α production and action, and shear stress (Figure 18.1) are appropriate candidates for study in human disease.

The role of transjugular intrahepatic portosystemic shunts (TIPS) as a therapeutic option for HPS remains undefined and should be considered an experimental treatment. Case reports have documented improved oxygenation in patients with HPS but interpretation of the results of TIPS placement are limited because of the short duration of follow-up, coexistence of other potential explanations of hypoxemia (i.e., hydrothorax), persistent post-TIPS intrapulmonary shunting, and small numbers of patients (61,62,63,64,65).

Liver transplantation is the only proved therapy for HPS based on the total resolution or significant improvement in gas exchange postoperatively in more than 85% of reported patients (66). However, the length of time for arterial hypoxemia to normalize after transplantation varies and may be delayed for over 1 year (67). In addition, mortality is increased after transplantation in patients with HPS compared to subjects without HPS, especially in those with severe HPS (66,68), and unique postoperative complications including pulmonary hypertension (69), cerebral embolic hemorrhages (70), and immediate postoperative deoxygenation requiring prolonged mechanical ventilation (71) have been reported. Prospective evaluation of the utility of the severity of HPS as a predictor of outcome after liver transplantation revealed that a preoperative Pao_2 of 50 mm Hg or less alone or in combination with a macroaggregated albumin shunt fraction of 20% or less were the strongest predictors of postoperative mortality (68). Innovative approaches such as frequent body repositioning (72) or inhaled NO (73,74) have been used to improve

postoperative gas exchange. Further research focused on the perioperative medical management of patients with HPS is needed to optimize survival.

Portopulmonary Hypertension

Definition

POPH is defined as a mean pulmonary artery pressure greater than 25 mm Hg and a pulmonary capillary wedge pressure less than 15 mm Hg occurring in the setting of portal hypertension (75). An elevated transpulmonary gradient (mean pulmonary artery pressure–pulmonary capillary wedge pressure >10 mm Hg) and/or pulmonary vascular resistance (>240 dyne sec/cm^{-5}) are additional criteria included in the definition of this syndrome.

Epidemiology

Changes consistent with pulmonary hypertension were found in 0.73% of patients with cirrhosis compared to a prevalence of 0.13% in subjects without chronic liver disease in a series of 17,901 autopsies (76). A subsequent prospective study of 507 patients who underwent right-sided heart catheterization (RHC) revealed a 2% prevalence of POPH (77). More recently, retrospective studies in liver transplantation candidates have found a prevalence ranging from 3.5% to 16% (78,79,80,81). The prevalence and severity of POPH do not appear to correlate with the degree of hepatic dysfunction or the severity of portal hypertension (77); however, the severity of cardiopulmonary symptoms worsens with increasing pulmonary hypertension (82). POPH has been described most commonly in patients with cirrhosis and portal hypertension, regardless of etiology, but has also been observed in disorders characterized by portal hypertension without cirrhosis (25).

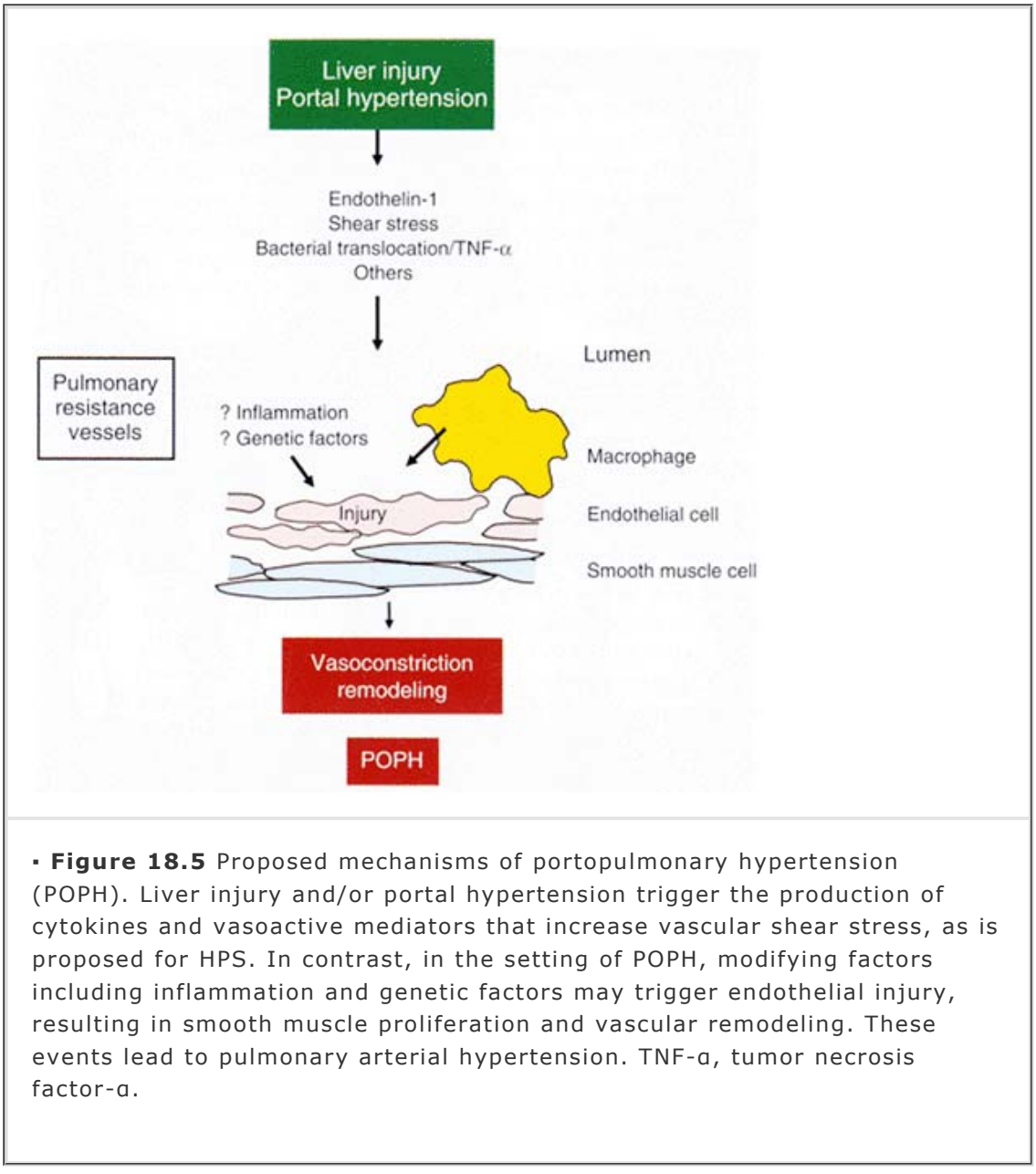
Pathology and Pathogenesis

The histologic features of POPH are similar to those seen in primary pulmonary hypertension. These include smooth muscle hypertrophy and hyperplasia, concentric intimal fibrosis, plexogenic arteriopathy, and necrotizing vasculitis (23,76,83,84,85). The underlying mechanisms in POPH remains incompletely understood and no animal models have been developed. Elevated portal pressure is critical for the development of pulmonary hypertension (77). The hyperdynamic circulatory state, causing increased vascular shear stress, and portosystemic shunting, causing altered production or metabolism of vasoactive substances, have been hypothesized to contribute to vascular changes in POPH (25). Endothelial and circulating factors (e.g., prostacyclin, thromboxane, serotonin, and ET-1) and polymorphisms in genes regulating vascular proliferation (e.g., serotonin and transforming growth factor-β [TGF-β] receptor superfamily) might contribute to POPH. One hypothesis suggests that endothelial injury or dysfunction may be a key early event that contributes to vascular proliferation and inflammation, leading to POPH (Fig. 18.5). In addition, HPS may coexist with POPH, suggesting that these two entities may share underlying pathogenetic mechanisms. One unifying concept is that the degree of endothelial dysfunction or injury may determine whether vascular proliferation and inflammation or vasodilatation occurs, with more severe injury leading to POPH, whereas less severe disease leads to HPS.

Clinical Features

Symptoms of POPH are nonspecific. Most patients may be asymptomatic (81). The most common symptom described in POPH is dyspnea on exertion, with the development of progressive fatigue, dyspnea at rest, peripheral edema, syncope, and chest pain as severity increases (86). Physical examination reveals jugular

venous distention, a loud pulmonary component of the second heart sound, and a systolic murmur resulting from tricuspid regurgitation. Lower extremity edema is commonly noted. Electrocardiographic abnormalities are similar to those seen in primary pulmonary hypertension and include evidence of right atrial enlargement, right ventricular hypertrophy, right-axis deviation, and/or right bundle branch block. Radiographic findings are generally subtle, but in advanced cases, a prominent main pulmonary artery or cardiomegaly due to prominent right cardiac chambers may occur. Gas exchange abnormalities are generally mild and less severe than those in HPS. An increased PAO_2–PaO_2 with mild hypoxemia and hypocarbia may be seen, particularly in more severe disease (25,87).



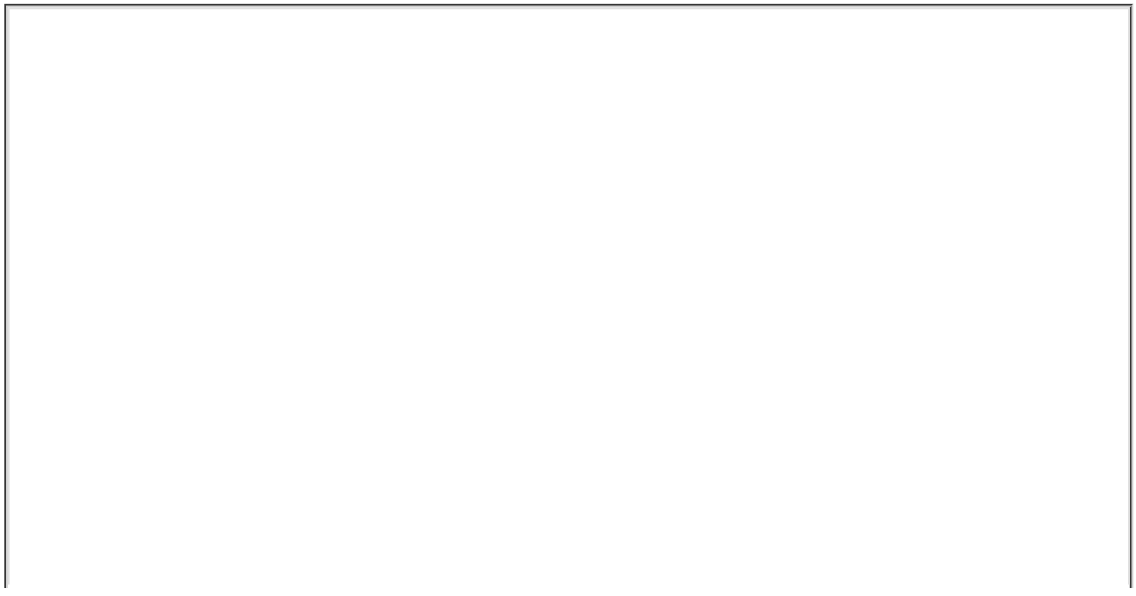
▪ **Figure 18.5** Proposed mechanisms of portopulmonary hypertension (POPH). Liver injury and/or portal hypertension trigger the production of cytokines and vasoactive mediators that increase vascular shear stress, as is proposed for HPS. In contrast, in the setting of POPH, modifying factors including inflammation and genetic factors may trigger endothelial injury, resulting in smooth muscle proliferation and vascular remodeling. These events lead to pulmonary arterial hypertension. TNF-α, tumor necrosis factor-α.

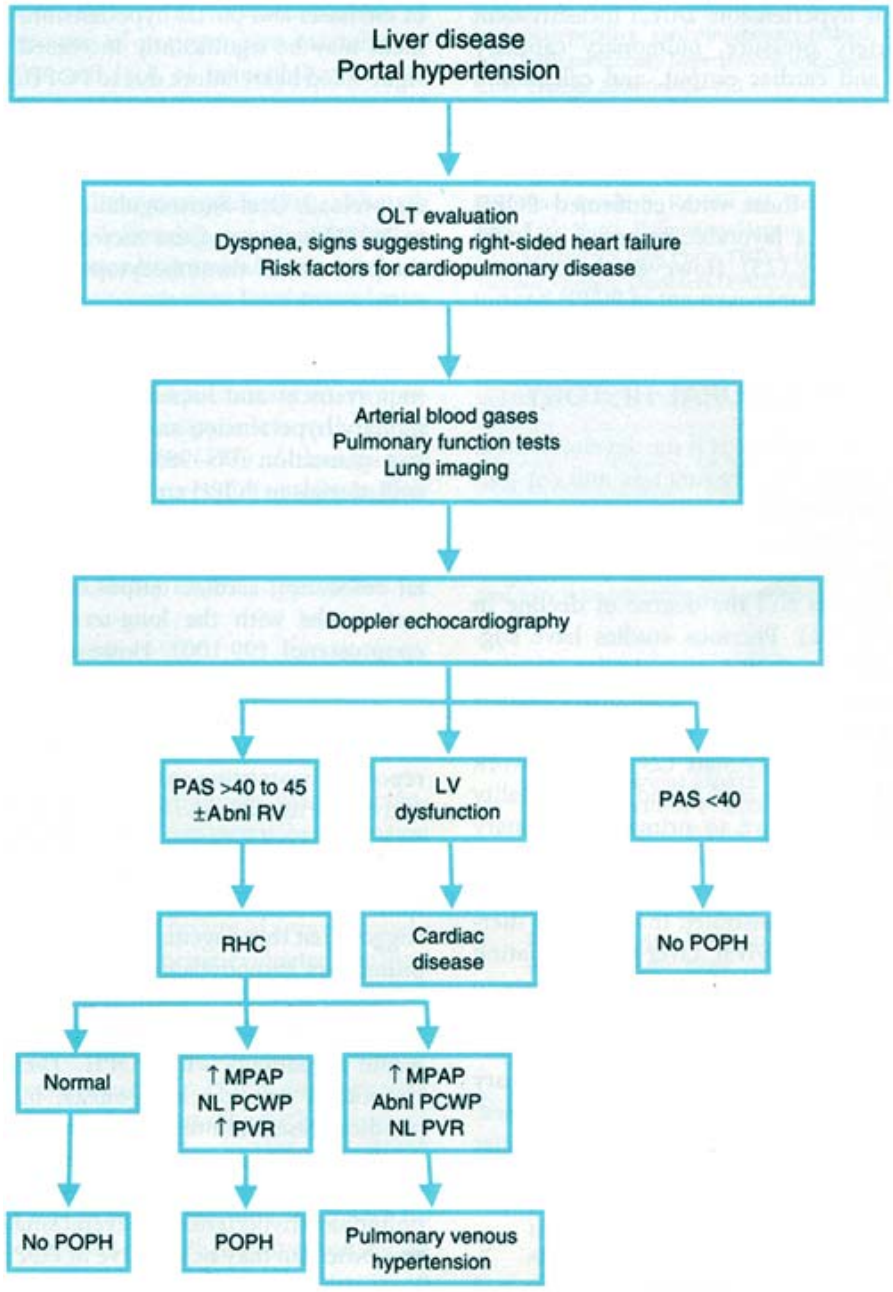
Evaluation for Portopulmonary Hypertension

Patients with POPH are often asymptomatic and the diagnostic utility of various clinical features is low (79,88). Therefore, the diagnosis of POPH requires a high index of suspicion (Fig. 18.6). Other causes of elevated pulmonary pressures and/or right-sided heart failure including left ventricular dysfunction, volume overload, and chronic obstructive lung disease have to be excluded. In general, in patients not being evaluated for liver transplantation, the presence of suggestive symptoms and signs and screening for POPH after exclusion of other cardiopulmonary diseases are reasonable. In all patients being evaluated for liver transplantation, regardless of signs or symptoms, screening is warranted because the presence of POPH may influence transplantation candidacy (89).

Transthoracic Doppler echocardiography is the best noninvasive screening study. If combined with intravenous contrast injection, screening for HPS and POPH can be accomplished at the same time. The presence of pulmonary hypertension is suggested by an increased estimated pulmonary artery systolic (PAS) pressure (derived from measuring the velocity of the tricuspid regurgitant jet), pulmonary valve insufficiency, right atrial enlargement, and/or right ventricular hypertrophy or dilatation. Several recent studies have evaluated the utility of estimated pulmonary arterial systolic pressure measurements in the diagnosis of POPH (81,88,90). In these studies, estimated PAS pressures used to define an elevated value ranged from 30 to 50 mm Hg. According to these studies, between 10% and 15% of patients had elevated estimated PAS

pressures by echocardiography, and roughly half of these patients were confirmed to have POPH. In a recent prospective study, Doppler echocardiography had positive and negative predictive values of 59% and 100%, respectively, in detecting POPH (81). However, the precise methods for estimating PAS pressures have not been standardized between studies and may have influenced the operating characteristics of echocardiographic screening. From a practical perspective, using an estimated PAS pressure of greater than 40 to 45 mm Hg to trigger further evaluation, particularly if right atrial and/or right ventricular abnormalities are also present, is likely to detect almost all cases of POPH. Most false-positive results occur commonly in the setting of elevated pulmonary venous pressures because of the hyperdynamic circulatory state and volume overload in cirrhosis (81).





▪ **Figure 18.6** Diagnostic approach to portopulmonary hypertension (POPH). See text for details. OLT, orthotopic liver transplantation; PAS, estimated pulmonary artery systolic pressure; Abnl, abnormal; RV, right ventricle; LV, left ventricle; RHC, right-heart catheterization; MPAP, mean pulmonary artery pressure; NL, normal; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance.

If echocardiographic findings suggest POPH, right-sided heart catheterization is indicated to confirm elevated pulmonary artery pressures and exclude

pulmonary venous hypertension. Direct measurement of pulmonary artery pressure, pulmonary capillary wedge pressure, and cardiac output, and

calculation of systemic and pulmonary vascular resistance, are included. Vasodilator responsiveness with a number of agents, most frequently NO and/or epoprostenol, may be undertaken in those with confirmed POPH in an effort to predict a favorable response to long-term vasodilator therapy (25). However, the utility of vasodilator testing in the management of POPH has not been studied.

Prognosis and Natural History

The major complication of POPH is the development of progressive right ventricular dysfunction and cor pulmonale. Survival in pulmonary hypertension correlates with the severity of right-sided cardiac dysfunction, as assessed by the degree of elevation in the right-sided cardiac pressures and the degree of decline in the cardiac output (91). Previous studies have suggested that survival in POPH was similar or even prolonged compared to primary pulmonary hypertension, possibly related to the beneficial effects of the hyperdynamic circulatory state (24). Recent work has challenged this concept and found that mortality was higher in POPH relative to primary pulmonary hypertension despite a higher cardiac index and lower systemic and pulmonary vascular resistance (92). To date, no studies have demonstrated that medical therapy for POPH improves survival. Liver transplantation in patients with mild POPH (<35 mm Hg) appears to have comparable outcomes relative to patients without pulmonary hypertension, although long-term follow-up has not been reported. At higher mean pulmonary artery pressures, perioperative mortality is increased, particularly in those with higher pulmonary vascular resistance values or lower cardiac output (89).

Therapy

Medical treatment for POPH improves symptoms and is largely based on experience with primary pulmonary hypertension. Treatment with vasodilators is the mainstay of therapy and can reverse the vasoconstriction associated with POPH, but it has little or no effect on the fibrotic and proliferative remodeling changes. In primary pulmonary hypertension, administration of calcium channel blockers prolongs survival (93), but these agents have not been studied in POPH because of the possibility that they may increase portal pressure (94). A single case has found short- and long-term beneficial pulmonary hemodynamic effects with the use of isosorbide-5’-mononitrate (95). The use of β-adrenergic blockers in patients with POPH is controversial on the basis of the potential risk for cardiac depression. Diuretics are often required to control fluid retention in cirrhosis and portal hypertension, and this requirement may be significantly increased in the setting of right-sided heart failure due to POPH. However, diuretics should be used with particular caution in POPH because intravascular volume depletion may critically reduce the cardiac output by decreasing right ventricle preload. Oral anticoagulation is not recommended in POPH because of the increased risk of bleeding in the presence of thrombocytopenia, coagulopathy, and gastroesophageal varices.

Prostacyclin (epoprostenol) is a potent vasodilator and platelet aggregation inhibitor that results in clinical improvement and increased survival in primary pulmonary hypertension and is useful as a bridge to lung transplantation (96,97,98). Although randomized, controlled trials in POPH are not available, two small series and a case report have also demonstrated improved mean pulmonary artery pressures, pulmonary vascular resistance, cardiac output, and 6-minute walking test results with the long-term use of intravenous epoprostenol (99,100).

However, epoprostenol does not appear to improve survival in POPH and whether it might provide a bridge to liver transplantation has not been clearly defined (101). In addition, there have been reports of worsening splenomegaly and hypersplenism and concerns related to worsening ascites with the long-term use of epoprostenol (102). Preliminary reports of the use of other prostacyclin analogs including treprostinil (subcutaneous injection) and iloprost (inhaled) suggest that these agents may also be useful to improve pulmonary hemodynamics in POPH.

Newer agents developed or under study for the treatment of primary pulmonary hypertension may also be useful in patients with POPH. These agents include endothelin receptor antagonists, inhaled NO, phosphodiesterase inhibitors, and L-arginine. Bosentan is an orally available dual ET receptor antagonist (A and B) that improves pulmonary hemodynamics in primary pulmonary hypertension. Several small studies support that bosentan may be effective in POPH (87,103,104,105). However, this agent has been associated with increases in hepatic enzymes (106), possibly because of inhibition of hepatocyte bile acid transport (107), and may lower systemic blood pressure. The safety of this agent in cirrhosis, particularly if advanced, is unknown. Sitaxsentan, a selective ET-A receptor blocker, has been associated with severe cases of acute hepatitis and should be avoided in POPH (108). Finally, two recent reports have shown beneficial hemodynamic effects of sildenafil in POPH without detrimental effects on systemic hemodynamics (109,110). The safety and efficacy of newer agents for POPH need to be established.

The efficacy of liver transplantation as a mode of treatment of POPH also remains controversial. On the basis of retrospective data and clinical experience, moderate to severe POPH (mean pulmonary artery

pressure >50 mm Hg) is a contraindication to transplantation because of perioperative mortality of approximately 40% and lack of reversibility of pulmonary hypertension (111,112). Patients with mild POPH (mean pulmonary artery pressure <35 mm Hg) appear to have no increase in perioperative cardiopulmonary mortality after liver transplantation, although the results of long-term follow-up and documentation of resolution of pulmonary hypertension has not been undertaken (89). The outcome after liver transplantation in patients with intermediate-severity POPH (mean pulmonary artery pressure 35 to 50 mm Hg) and in those who have improvement in pulmonary artery pressures on long-term medical therapy is less well defined and requires further evaluation (111). Although case reports have demonstrated successful outcomes after combination lung–liver or heart–lung–liver transplantation, limited organ availability and technical challenges limit the feasibility of such approaches for POPH (113).

Summary and Conclusions

HPS and POPH are unique pulmonary vascular complications of liver disease and/or portal hypertension that may cause significant morbidity and influence survival and liver transplantation candidacy. HPS occurs in approximately 20% and POPH occurs in approximately 6% of patients with cirrhosis being evaluated for liver transplantation. Dilatation in the pulmonary microvasculature is an important event that leads to hypoxemia and symptoms in HPS. Vasoconstriction and remodeling in resistance vessels occur in POPH and may lead to right-sided cardiac dysfunction. The pathogenesis of pulmonary vascular abnormalities in HPS and POPH is an area of ongoing investigation, and similar mechanisms may

play a role in each syndrome. There are no effective medical therapies for HPS, but liver transplantation can reverse the syndrome in most patients. In contrast, there are symptomatic medical therapies for POPH, but for many patients liver transplantation is currently contraindicated or controversial. Transplantation carries increased mortality in both severe HPS and POPH, underscoring the importance of screening for these disorders in patients undergoing liver transplantation evaluation.

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These two studies support that the presence of HPS increases mortality in patients with cirrhosis. The second study also supports that liver transplantation improves survival in patients with HPS and cirrhosis.

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Chapter 19

Ascites and Spontaneous Bacterial Peritonitis

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Miguel Navasa

Key Concepts

- The most common cause of ascites in humans is cirrhosis followed by congestive heart failure, malignant ascites, and tuberculous peritonitis. Measurement of serum to ascitic fluid gradient of albumin and the concentration of leukocytes, standard cytologic examination for malignant cells, measurement of the concentration of adenosine deaminase, and the detection of deoxyribonucleic acid of *Mycobacterium* organisms by means of polymerase chain reaction of ascitic fluid are the specific tests for the differential diagnosis of the causes of ascites. However, investigation of physical and exploratory findings characteristic of these entities are also important in making the diagnosis.
- In cirrhosis the ascitic fluid protein concentration is considerably lower than the plasma protein concentration. This is due to the capillarization of the hepatic sinusoids, which reduces their permeability to plasma proteins, and to a major contribution of the splanchnic microcirculation (with low permeability to proteins) to the formation of ascites. The concentration of proteins in ascitic fluid in cirrhosis correlates inversely with the degree of portal hypertension.
- Lymph formation within the liver and the splanchnic circulation is markedly increased in patients with nonascitic cirrhosis who have portal hypertension. Lymph is effectively transported into the systemic circulation through the splanchnic lymphatic system and thoracic duct. Ascites develops when lymph formation overcomes the transport capacity of the lymphatic system. In the splanchnic organs (e.g., intestines, stomach, peritoneum) the increased lymph formation is more related to an increased blood inflow into the splanchnic microcirculation secondary to an arterial vasodilatation, which leads to an increase in capillary pressure and permeability, than to a backward transmission of the increased portal venous pressure into the splanchnic capillaries. The mechanism of the splanchnic arterial vasodilatation in cirrhosis is related to portal hypertension, which increases local production of vasodilatory substances, particularly nitric oxide.
- Splanchnic arterial vasodilatation is the key mechanism of ascites formation (forward theory of ascites). In addition to increasing lymph formation in the splanchnic microcirculation, splanchnic arterial vasodilatation impairs arterial circulatory function and leads to the activation of the renin–

aldosterone system, sympathetic nervous system, and antidiuretic hormone and renal sodium and water retention. The simultaneous occurrence of excessive lymph formation and renal retention of fluid lead to continuous ascites formation.

- There is an alteration in cardiac function in cirrhosis that could also contribute to the pathogenesis of the circulatory and renal dysfunction and to the formation of ascites. Although higher than normal in most patients, cardiac output decreases during the course of decompensated cirrhosis despite the reduction in peripheral vascular resistance. On the other hand, the heart rate does not increase in response to the progressive stimulation of the sympathetic nervous system, indicating impairment of cardiac chronotropic function.
- Moderate sodium restriction (90 mmol/day), spironolactone, and furosemide are the basis of the medical management of ascites. Spironolactone is the principal drug. Furosemide can be added to spironolactone to increase diuretic response. Medical treatment is indicated in patients with moderate ascites.
- Therapeutic paracentesis with intravenous administration of albumin (8 g/L of ascitic fluid removed) is the treatment of choice for tense ascites in cirrhosis. Sodium restriction and diuretics should be used to prevent the reaccumulation of ascitic fluid. Large-volume paracentesis without plasma volume expansion frequently impairs circulatory function, which although asymptomatic can adversely influence the clinical course.
- Peritoneovenous shunting and transjugular intrahepatic portacaval shunting are effective therapies for refractory ascites in cirrhosis. They are, however, associated with a high rate of complications, particularly shunt obstruction, and do not improve the overall results of paracentesis in relation to the duration of hospitalization and survival. The recent introduction of covered stents with less rate of shunt obstruction will probably increase the indication of transjugular intrahepatic portacaval shunting in patients with refractory ascites.
- Spontaneous infection of the ascitic fluid (spontaneous bacterial peritonitis) is a frequent event in cirrhosis (10% to 30% prevalence in patients admitted to hospital with ascites). Its pathogenesis is multifactorial, including translocation of bacteria from the intestinal lumen into the circulation, impaired reticuloendothelial system phagocytic activity leading to sustained bacteremia, and decreased antibacterial activity of the ascitic fluid. The most important predictive factor of spontaneous bacterial peritonitis is a low ascitic fluid protein concentration (<10 g/L). The protein concentration in ascitic fluid correlates closely with the antibacterial activity of ascites.
- The gold standard method for the diagnosis of spontaneous bacterial peritonitis is the measurement of the concentration of polymorphonuclear leukocytes in ascitic fluid (diagnosis is made when it is >250 cells/mm^3). Leukocyte esterase reagent strips are useful for a rapid bedside diagnosis of spontaneous bacterial peritonitis. Cultures of ascitic fluid and/or blood cultures are positive in approximately 50% of cases. The organism most commonly isolated in spontaneous bacterial peritonitis is *Escherichia coli*.
- Third-generation cephalosporins are the best antibiotics for the empiric management of spontaneous bacterial peritonitis. The rate of resolution of the infection is more than 90%. However, despite rapid resolution of the infection, 30% of patients die during hospitalization.

- The most common cause of death in patients with spontaneous bacterial peritonitis is a multiorgan failure secondary to severe impairment of circulatory function. It is characterized by intense reduction of the cardiac output; aggravation of splanchnic arterial vasodilatation and portal hypertension; severe impairment of renal, hepatic and cerebral function; and a relative adrenal insufficiency. Circulatory support with intravenous administration of albumin at the time of diagnosis of infection reduces hospital mortality by 60%.
- The probability of recurrence of spontaneous bacterial peritonitis is extremely high (>60% at 1 year). Antibiotic prophylaxis with oral norfloxacin drastically reduces the rate of recurrence of spontaneous bacterial peritonitis. Norfloxacin is also used for primary prophylaxis of bacterial infection in the care of patients with cirrhosis and gastrointestinal hemorrhage and of those with ascites who are at high risk for a first episode of spontaneous bacterial peritonitis (patients with advanced liver disease and low ascitic fluid protein concentration).
- Spontaneous bacterial peritonitis caused by quinolone-resistant bacteria is emerging as a clinical problem.

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Ascites is the most common complication in patients with cirrhosis. It develops as a consequence of a severe impairment of liver function and portal hypertension, and, not surprisingly, it is associated with a poor prognosis. Great advances have been made in the pathogenesis and management of cirrhotic ascites. It is now evident that ascites formation in cirrhosis cannot be considered as a consequence of "backward" transmission of the increased intrahepatic hydrostatic pressure into the hepatic and splanchnic microcirculation and a decrease in intravascular oncotic pressure because of the impaired hepatic synthesis of albumin. Ascites formation is related more to events occurring in the arterial vascular compartment and in the kidneys than to those occurring in the portal venous system. The central event of ascites formation in cirrhosis is a splanchnic arterial vasodilatation secondary to portal hypertension. It simultaneously induces two different types of events: (a) A "forward" increase in capillary pressure because of a great inflow of blood at high pressure into the splanchnic microcirculation, which favors the leakage of fluid into the peritoneal cavity (1,2), and (b) impairment of systemic hemodynamics and renal function, which leads to sodium and water retention. The recent demonstration that cardiac output decreases during the course of cirrhosis in parallel with the progression of the splanchnic arterial vasodilatation (3) adds a new dimension to the complexity of the pathogenesis of circulatory dysfunction and ascites in chronic liver diseases.

These new concepts in the pathogenesis of ascites are important for a better understanding of several events occurring in patients with cirrhosis and ascites. Most important, they are the basis for the design of new treatments in these patients. For example, it is now well known that the systemic circulation is extremely unstable in patients with decompensated cirrhosis because of the resistance of the splanchnic arterial vascular compartment to the effect of endogenous vasoconstrictors (e.g., norepinephrine or angiotensin II). Therefore, the regulation of arterial blood pressure largely depends on the effect of these substances on the renal circulation. This explains why patients with cirrhosis and ascites are predisposed to the development of renal vasoconstriction and

hepatorenal syndrome (HRS) (4). HRS may develop spontaneously; however, it most commonly occurs in close chronologic relationship with an event that increases arterial vasodilatation and decreases cardiac function (e.g., therapeutic paracentesis or spontaneous bacterial peritonitis [SBP]) (5). The recent demonstration that HRS in cirrhosis can be successfully treated by the administration of vasoconstrictor agents associated with plasma volume expansion is the most outstanding consequence of the new concept of the circulatory dysfunction associated with ascites (6,7).

The introduction of the transjugular intrahepatic portacaval shunt (TIPS) and the progressive abandonment of the peritoneovenous shunting for the treatment of refractory ascites are the most relevant changes in therapy during the last decade (8,9). During this period, the important role of paracentesis in the management of patients with cirrhosis and tense ascites was clearly established (10). Also, the initial studies on a new family of drugs, the aquaretic V2 antagonists, was performed in patients with cirrhosis and ascites (11,12). These agents, by inhibiting the renal tubular effect of antidiuretic hormone, increase diuresis without affecting sodium excretion. The net effect is an increase in free water excretion and, in patients with hyponatremia, normalization of serum sodium concentration.

SBP, the spontaneous infection of ascitic fluid, is the clinical condition in hepatology with the most impressive improvement in prognosis. The hospital mortality rate has decreased from 80% in the early 1970s to 10% in the last randomized controlled trial published in 2000 (13). An early diagnosis and the use of effective non-nephrotoxic antibiotics were the initial factors improving prognosis. In this regard, the recent observation that leukocyte esterase reagent strips are

very sensitive and specific for the diagnosis of SBP will facilitate its rapid diagnosis at the bedside and an earlier treatment (14,15,16,17). For many years the hospital mortality rate associated with SBP ranged between 30% and 40% despite the resolution of the infection in more than 90% of the patients. Studies showing that SBP induces a deterioration of circulatory function, which may not be reversible after resolution of the infection and causes multiorgan failure, are essential to the understanding that circulatory support is important in patients with SBP (3,5,18,19). Subsequently, a randomized controlled trial clearly demonstrated that energetic plasma volume expansion with albumin at the time of diagnosis of the infection markedly reduces the incidence of circulatory and renal dysfunction and hospital mortality in patients with SBP (13). This positive finding, however, is counterbalanced by recent observations of patients with quinolone and trimethoprim–sulfamethoxazole–resistant SBP suggesting that these antibiotics are at present not as effective in the prophylaxis of SBP as it was reported in the past.

The aim of the current chapter is to review the pathogenesis, diagnosis, and treatment of ascites and SBP. Particular attention has been paid to these new advances in the field. Classical well-established concepts are more briefly summarized. The reader is referred to Chapter 17 for a better understanding of some aspects of the current chapter.

Ascites

Clinical Aspects

Etiology

Many diseases can lead to the accumulation of fluid within the peritoneal cavity. They can be grouped into two major categories depending on whether they directly affect the peritoneum (Table 19.1). In the first category, ascites forms as a consequence of primary or secondary peritoneal disease (e.g., tuberculous, fungal, parasitic, and granulomatous peritonitis; vasculitis; eosinophilic gastroenteritis; Whipple's disease; and primary or metastatic peritoneal tumor). The second category includes diseases causing sinusoidal portal hypertension (e.g., cirrhosis, acute alcoholic hepatitis, fulminant or subacute viral or toxic hepatitis, Budd-Chiari syndrome, hepatic veno-occlusive disease, congestive heart failure, constrictive pericarditis, and inferior vena caval obstruction over the liver) and hypoalbuminemia (e.g., nephrotic syndrome, protein-losing enteropathy, and malnutrition), and a variety of disorders that may cause ascites by different mechanisms (e.g., myxedema, benign and malignant ovarian tumors, ovarian hyperstimulation syndrome, pancreatitis, biliary tract leakage, chronic renal failure, and diseases affecting the lymphatic system of the splanchnic area). The common feature of all these diseases is that the peritoneum is not affected. By far the most

frequent cause of ascites is hepatic cirrhosis followed by neoplasm. Other relatively frequent causes are congestive heart failure and tuberculous peritonitis. These four conditions account for more than 90% of ascites in Europe and North America.

Table 19.1. Causes of Ascites

PORTAL HYPERTENSION
Cirrhosis
Alcoholic hepatitis
Fulminant hepatitis
Subacute hepatitis
Hepatic veno-occlusive disease
Massive liver metastasis
Congestive heart failure
Constrictive pericarditis
Budd-Chiari syndrome
MISCELLANEOUS DISORDERS
Myxedema
Ovarian disease
Carcinoma
Benign tumors
Ovarian hyperstimulation syndrome
Pancreatic ascites
Bile ascites
Chylous ascites
Nephrogenic ascites
Acquired immunodeficiency syndrome
HYPOALBUMINEMIA
Nephrotic syndrome

- Protein-losing enteropathy
- Malnutrition

PERITONEAL DISEASES

- Malignant ascites
  - Peritoneal mesothelioma
  - Peritoneal carcinomatosis
- Infectious peritonitis
  - Tuberculosis
  - *Chlamydia trachomatis*
  - Fungal and parasitic peritonitis
    - *Candida albicans*
      - *Histoplasma capsulatum*
      - *Coccidioides immitis*
    - *Cryptococcus neoformans*
      - *Schistosoma mansoni*
      - *Strongyloides stercoralis*
    - *Entamoeba histolytica*
- Other peritoneal diseases
  - Sarcoidosis
  - Starch granulomatous peritonitis
  - Barium peritonitis
    - Vasculitis
      - Systemic lupus erythematosus
      - Henoch-Schönlein purpura
    - Eosinophilic gastroenteritis
      - Whipple's disease

Detection of ascites

The diagnosis of ascites is simple when large amounts of fluid accumulate in the abdominal cavity. However, diagnosis can be difficult when the volume of ascitic fluid is small or if the patient is obese. In these circumstances, ultrasonography is the best method for the detection of ascites because it is not expensive and gives information about the liver and other intra-abdominal organs. Ascites due to portal hypertension characteristically appears as homogeneous, echo-free areas surrounding and interposed between the loops of bowel and viscera in a relatively uniform manner. When the amount of ascites is small, the fluid tends to collect in the flanks and the superior right paracolic gutter, around the liver, and in the lower peritoneal reflection in the pelvis. Atypical sonographic characteristics, such as the presence of multiple echoes, septations or fibrous strands within the ascitic fluid, and loculation of fluid, are highly suggestive of an ascites unrelated to portal hypertension. The sonographic characteristics of the liver, suprahepatic veins, portal venous system, peritoneum, spleen, stomach, intestine, and intra-abdominal lymphatic nodes, are of great help in assessing the etiology of ascites.

Characteristics of cirrhotic ascites

The biochemical and cytologic characteristics of the ascitic fluid provide important information for the differential diagnosis. The ascitic fluid in cirrhosis

is characteristically transparent and yellow/amber in color. In most patients (70%) the total protein concentration is lower than 2.5 g/dL and approximately 50% correspond to albumin. The total protein concentration in ascitic fluid correlates inversely with portal pressure. It decreases during the course of the disease as portal hypertension increases (20,21). SBP characteristically develops in patients with a low total protein concentration in ascitic fluid (<1 g/dL) (22). Although it has been suggested that this may be due to a low concentration of proteins with antibacterial activity in the ascitic fluid, an alternative explanation is that patients with low total ascitic protein concentration are those with higher portal hypertension and greater liver insufficiency and, therefore, at higher risk for developing bacterial infections.

The ascitic fluid in most patients with cirrhosis without SBP has a concentration of leukocytes lower than 300 to 500 μL/mm^3 (usually <100 μL/mm^3). In some cases, however, it can be higher than 500 μL and even more than 1,000 μL. More than 70% of these white blood cells are mononuclear leukocytes. In contrast, in patients with SBP the ascitic fluid concentration of polymorphonuclear neutrophils (PMNs) is more than 250 μL (usually >2,000 μL). The ascitic fluid of patients with uncomplicated cirrhosis also has a high concentration of macrophages. The absolute concentration of leukocytes in ascitic fluid but not that of PMNs increases during diuretic treatment.

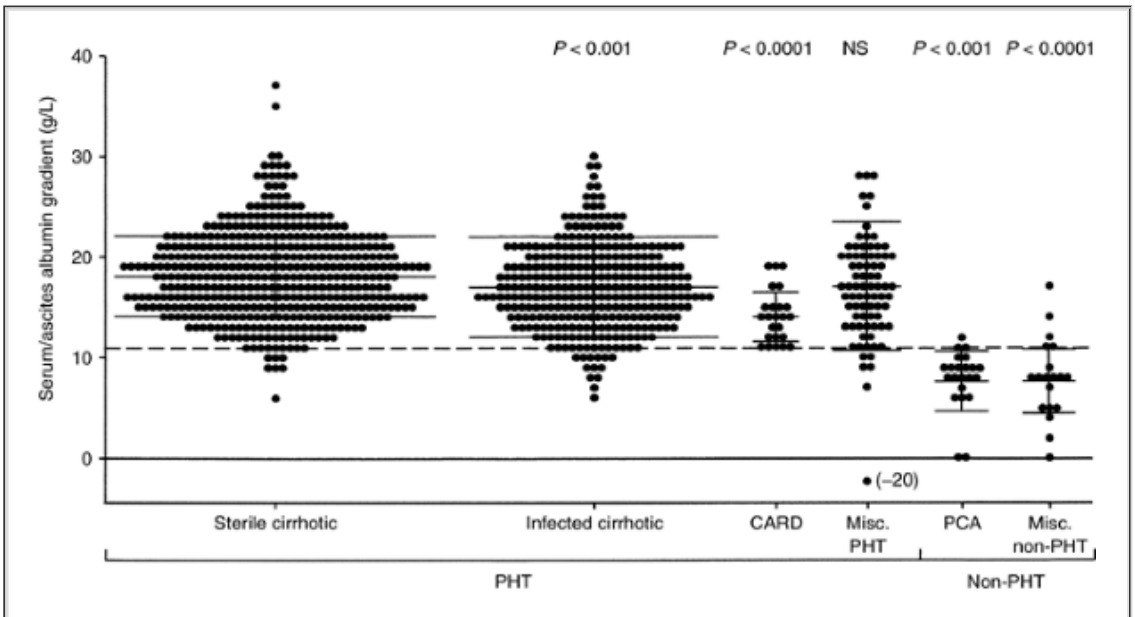
The concentration of red blood cells in cirrhotic ascites is usually less than 1,000 μL, although it can be higher. Bloody ascites (hematocrit >0.50) occurs in 2% of patients. In some cases, bloody ascites is secondary to a superimposed superficial hepatocellular carcinoma bleeding into the peritoneal cavity. In most cases no apparent cause can be detected. Because hepatic and thoracic lymph is often bloody in cirrhosis, bloody ascites can be caused by leakage of bloody lymph into the abdominal cavity.

Ascites in cirrhosis has traditionally been considered an inert fluid mainly composed of water, electrolytes, and proteins that transudates passively from the microvascular compartment into the peritoneal cavity. At present, there is evidence that many metabolic reactions and synthetic processes occur within the ascitic fluid. For example, in patients with cirrhosis a complex coagulation process within the ascitic fluid results in intraperitoneal coagulation and primary and secondary fibrinolysis. The macrophages of ascitic fluid synthesize vasodilatatory substances (e.g., nitric oxide, adrenomedullin, vascular endothelial growth factor), a feature not observed in their precursors, the circulating monocytes (23). The pathophysiologic significance of this finding is unknown. It is also unknown whether this reflects a generalized activation of the peritoneal macrophages or a local activation by some factor within the ascitic fluid (e.g., an endotoxin). The concentration of interleukin-6 and tumor necrosis factor is higher in ascites than in plasma, indicating a local production of cytokines (19). Also, the concentration of leptin and vascular endothelial growth factor is higher in ascitic fluid than in plasma (24,25). The angiogenic activity of the ascitic fluid of patients with cirrhosis may be related to this feature (26). Finally, the ascitic fluid has antibacterial activity, which correlates directly with the total ascitic fluid protein concentration (21). Substances such as complement, fibronectin, cytokines, and nitric oxide are implicated in this effect, which may be an important defensive mechanism against SBP. Not surprisingly, the infusion of ascitic fluid within the general circulation is associated with important biologic effects, the most important being intravascular coagulation and fever.

Differential diagnosis of cirrhotic ascites and other types of ascites

Malignant ascites is macroscopically bloody in only 10% of patients.
Differentiation from cirrhotic ascites

is based mainly on the characteristics of the ascitic fluid and on additional diagnostic findings. The total ascitic protein concentration is over 3.0 g/dL in most patients with malignant ascites. The serum to ascitic fluid gradient of albumin (usually <1.1 in malignant ascites and higher in cirrhotic ascites) is more accurate than total protein concentration in ascitic fluid for the differentiation of cirrhotic versus malignant ascites (18) (Fig. 19.1). The concentration of lactate dehydrogenase and of cholesterol in malignant ascites is higher than the corresponding values in plasma and than values observed in cirrhotic ascites. However, these values do not improve the diagnostic sensitivity of the serum to ascitic fluid albumin gradient. Conventional cytologic examination is 60% to 90% accurate in the diagnosis of malignant ascites if adequate volumes of fluid (several hundred milliliters) and concentration techniques are used. Immunocytochemical techniques with monoclonal or polyclonal antibodies against tumor markers may help differentiate malignant cells from atypical mesothelial cells. Laparoscopy and direct biopsy of peritoneal lesions may be necessary to confirm the diagnosis in the patients with negative cytologic results.



▪ **Figure 19.1** Serum/ascites albumin gradient in patients with portal hypertension (PHT) (sterile cirrhotic, infected cirrhotic, cardiac failure [CARD], and miscellaneous [Misc. PHT]) and in nonportal hypertensive (non-PHT) patients (peritoneal carcinomatosis [PCA] and miscellaneous nonportal hypertension [Misc. non-PHT]). (From Runyon BA, Montano AA, Akriviadis EA, et al. The serum-ascites albumin gradient is superior to the exudate-transudate concept in the differential diagnosis of ascites. *Ann Intern Med* 1992;117:218, with permission.)

Patients with ascites secondary to postsinusoidal portal hypertension (e.g.,

congestive heart failure, constrictive pericarditis, obstruction of the inferior vena cava, Budd-Chiari syndrome) also have a high total ascitic fluid albumin ratio less than 1.1. Lactate dehydrogenase and cholesterol concentrations, however, are not increased. The diagnosis of congestive heart failure and acute Budd-Chiari syndrome is easy from a clinical point of view. However, differentiation between cirrhotic ascites and that secondary to constrictive pericarditis or chronic Budd-Chiari syndrome can be difficult. Patients with ascites due to constrictive pericarditis often lack symptoms of congestive heart failure. On the other hand, in patients with chronic Budd-Chiari syndrome, the protein concentration in ascitic fluid may be low because of capillarization of the hepatic sinusoids (see later) and hepatic stigmata, abnormal liver function test results, splenomegaly, and esophageal varices. It is, therefore, essential to seek physical and additional diagnostic findings characteristics of these entities. This often requires the performance of standard radiologic examination, electrocardiography, and cardiac echography for pericarditis and hepatic ultrasonography or computed tomography to visualize the major hepatic veins in Budd-Chiari syndrome. In many cases, however, diagnosis can only be achieved after cardiac or hepatic venous catheterization and liver biopsy. Ascites due to postsinusoidal portal hypertension secondary to deficient venous drainage is occasionally seen in the postoperative period of liver transplantation (27).

The differential diagnosis between cirrhotic ascites and ascites secondary to tuberculous peritonitis is important because alcoholic cirrhosis can predispose

to peritoneal tuberculosis. Tuberculous peritonitis is frequent among patients with cirrhosis and acquired immunodeficiency syndrome. Ascites due to tuberculous peritonitis is characterized by an increased concentration of proteins (>3 g/dL) and lymphocytes. However, in cirrhosis with ascites and tuberculous peritonitis the ascitic fluid may be a transudate. The concentration of adenosine deaminase, an enzyme that participates in the proliferation and differentiation of lymphocytes, is increased in tuberculous pleural effusions and ascitic fluid in tuberculous peritonitis. It has been reported, however, that the percentage of false-negative results in tuberculous peritonitis is high in the presence of cirrhosis (28). The diagnosis of tuberculous peritonitis cannot be based on cultures of ascitic fluid because the usual techniques of culturing acid-fast bacilli requires several weeks of incubation and frequently gives false-negative results (21). On the other hand, although it has been suggested that the proportion of positive culture results may be as high as 80% when 1L of ascitic fluid is concentrated by means of centrifugation, the proportion reported in most studies is much lower. The detection of deoxyribonucleic acid (DNA) of *Mycobacterium tuberculosis* by means of polymerase chain reaction (PCR) assay of ascitic fluid is rapid and appears to be as sensitive as culture. False-negative results, however, have been reported, justifying the administration of antituberculosis treatment in patients with clinical and histologic features characteristic of peritoneal tuberculosis, even in cases with negative results from culture and PCR analysis. Laparoscopy and direct biopsy of the affected areas is required for the diagnostic confirmation and differentiation from other conditions causing granulomatous peritonitis (e.g., sarcoidosis, Crohn's disease).

Chylous ascites consists of a macroscopically turbid and milky ascites caused by a high concentration of chylomicrons rich in triglycerides (29). The principal causes of chylous ascites in adults are primary abnormalities of the lymphatic vessels (lymphangiectasia) and obstruction of the lymphatic system by neoplasms, particularly lymphoma. Chylous ascites should be differentiated from

pseudochylous ascites, in which, although the macroscopic appearance is identical, the triglyceride concentration is less than 110 mg/dL (the diagnostic cutoff for chylous ascites). Cirrhosis is an infrequent cause of chylous ascites that is usually related to hydrostatic hypertension within the splanchnic lymph vessels, which can lead to spontaneous rupture of some of these vessels into the abdominal cavity. Other causes of chylous ascites are surgical procedures involving the retroperitoneal region (including splenorenal shunt), pancreatitis, sarcoidosis, tuberculosis, and abdominal trauma.

Biliary and pancreatic ascites are caused by leakage of bile and pancreatic fluid, respectively, into the abdominal cavity. In biliary ascites, paracentesis yields a green ascitic fluid with a concentration of bilirubin considerably higher than that in plasma. Although bile leakage into the abdominal cavity can induce signs and symptoms of biliary peritonitis, some patients have no symptoms other than the accumulation of a large amount of biliary ascitic fluid. Therefore, biliary ascites should be considered a possible diagnosis when any patient accumulates intra-abdominal fluid after liver biopsy or biliary surgery (30). Pancreatic ascitic fluid is usually an exudate (ascitic fluid protein concentration is generally >3 g/dL), contains very high concentrations of pancreatic enzymes, and is mainly secondary to chronic pancreatitis. Because most patients with this disorder have alcoholism and may have massive ascites with little or no abdominal pain, the differential diagnosis of pancreatic from cirrhotic ascites can be difficult on clinical grounds (24).

Peripheral edema and cirrhotic hydrothorax

Edema in the lower extremities is frequent in patients with cirrhosis. In many cases it precedes the development of ascites by weeks or months. It can also appear simultaneously with the onset of ascites, or weeks or months thereafter. Hypoalbuminemia and increased venous pressure in the lower extremities due to constriction of the intrahepatic segment of the inferior vena cava or due to the high intra-abdominal pressure caused by the presence of ascites have been proposed as possible mechanisms. Massive peripheral edema with minimal or no ascites is found in patients with cirrhosis having severe hepatic insufficiency and low portal hypertension from TIPS insertion.

Five percent patients with cirrhosis have pleural effusion in the absence of pulmonary or pleural diseases or any other potential cause of hydrothorax. Clinical ascites is almost always evident, and the pleural effusion is usually right sided. The mechanism of this cirrhotic hydrothorax in most cases is the direct passage of ascites through defects in the diaphragm into the pleural space. The driving force is the hydrostatic gradient between the positive intra-abdominal pressure and the negative intrathoracic pressure. In cases of cirrhotic hydrothorax without detectable abdominal ascites (thoracic ascites) the passage of fluid into the pleural cavity probably equals the rate of ascites formation. Because ascites and the pleural fluid of cirrhotic hydrothorax have the same origin, a different cause of pleural effusion should be suspected if marked differences in biochemical and cytologic characteristics are observed between both fluids. Because cirrhotic hydrothorax occurs with abdominal ascites, patients with this condition may

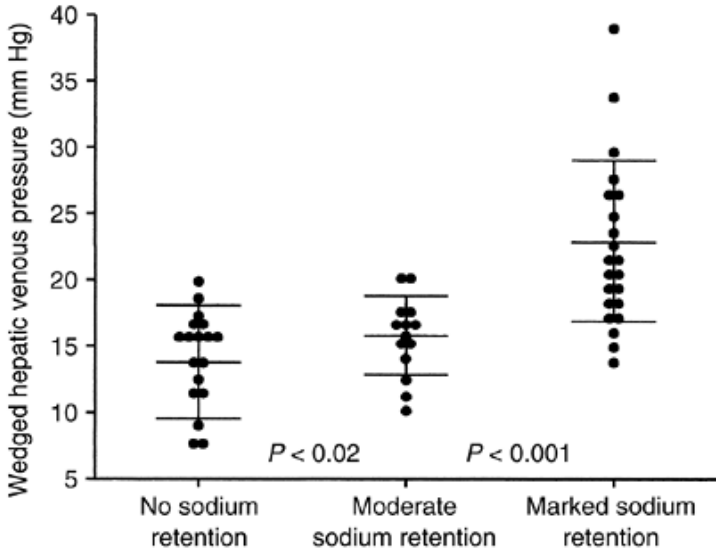
contract spontaneous infection of the pleural fluid (spontaneous bacterial empyema) (31).

Local Intra-Abdominal Factors in the Formation of Ascites

Portal hypertension and leakage of fluid from the intravascular compartment to the peritoneal cavity

There is substantial evidence that severe portal hypertension is the main disorder in the formation of ascites in cirrhosis. Patients have significantly higher portal pressure than those without ascites (Fig. 19.2). Ascites develops only when the hepatic venous pressure gradient (an estimation of the intrahepatic vascular resistance) is more than 12 mm Hg. Ascites is unusual in patients treated with a surgical side-to-side or end-to-side portacaval shunt for bleeding varices. In patients treated by TIPS, ascites frequently disappears after insertion of the stent and reappears if there is malfunction of the shunt.

Ascites is a frequent complication of diseases associated with increased hydrostatic pressure in the hepatic sinusoids (diseases that cause postsinusoidal blockage of the hepatic blood flow such as pericarditis, congestive heart failure, suprahepatic vena caval obstruction, Budd-Chiari syndrome, and hepatic veno-occlusive disease) and of those in which the blockade of the hepatic blood flow occurs mainly at the sinusoidal level (e.g., cirrhosis, severe acute alcoholic hepatitis, and fulminant or subacute toxic or viral hepatitis). Ascites is unusual in diseases associated with intrahepatic or extrahepatic presinusoidal portal hypertension. On the basis of these features and the results of early experimental studies, it has been traditionally considered that ascites is derived mainly from the hepatic microcirculation. Although differences in permeability characteristics between the hepatic and the splanchnic peritoneal (gastric and intestinal) microcirculation also support this concept, recent data suggest that ascites in cirrhosis is derived from both the hepatic and the splanchnic microcirculation (1).

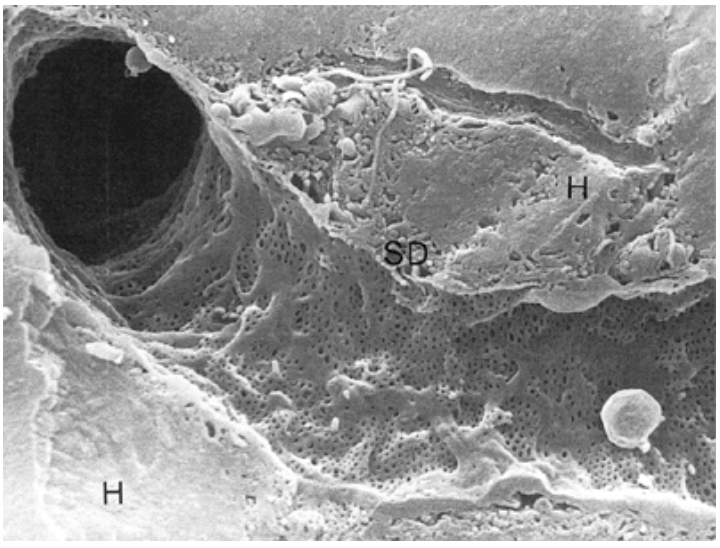


▪ **Figure 19.2** Wedged hepatic venous pressure in patients with compensated cirrhosis (no ascites) and in those with ascites and moderated and marked sodium retention. (From Bosch J, Arroyo V, Betriu A, et al. Hepatic hemodynamics and the renin-angiotensin system in cirrhosis. *Gastroenterology* 1980;78:92–99, with permission.)

Transmicrovascular fluid exchange in postsinusoidal and prehepatic portal hypertension

The hepatic sinusoids do not have basement membranes. They are lined only by endothelial cells, Kupffer cells, and stellate (fat-storing or Ito) cells. The endothelial cells are by far the main component of the sinusoidal wall. Kupffer cells also contribute to the sinusoidal wall, although they are most often within the sinusoidal lumen attached by processes to the endothelial cells. The endothelial cells form a porous sinusoidal wall, with apertures ranging between 100 and 500 nm in radius (Fig. 19.3). Microvilli from the hepatocytes cross the space of Disse and pass through these pores to reach the sinusoidal lumen. Stellate cells, together with few collagen fibers and other particles, are mainly located in the space of Disse. Under normal conditions this is an inconspicuous space in free communication with the interstitial space of the portal and central venous area, where there are terminal lymphatic vessels. The characteristics of the sinusoidal wall explain why, in the normal liver, the concentration of proteins in the hepatic lymph is approximately 90% of that in plasma.

The trans-sinusoidal oncotic gradient in the microcirculation of a normal liver, therefore, is very low. In contrast, the splanchnic capillaries are much less porous (the estimated pore size is 50 to 100 times less than that of the hepatic sinusoids) and have a basement membrane. Not surprisingly, the concentration of protein is lower in the splanchnic than in the hepatic lymph (lymph to plasma ratio of proteins is 0.50 in the intestinal lymph vs. 0.85 in the hepatic lymph) (1,32,33).



▪ **Figure 19.3** Normal hepatic sinusoid in rat liver. The fenestrae are regularly distributed in the sieve plates, which are separated by intervening cytoplasmic processes. *H*, hepatocyte; SD, space of Disse. (From MacSween RNM, Scothorne RJ. Developmental anatomy and normal structure. In: MacSween RNM, Anthony PP, Scheuer PJ, et al. eds. *Pathology of the liver*,

3rd Ed. New York: Churchill Livingstone, 2002:1–66, with permission.)

There are other marked differences between the hepatic and the splanchnic microcirculation. First, capillary pressure is autoregulated in the splanchnic circulation but not in the liver. The acute increase in pressure in the hepatic veins (e.g., after the constriction of the suprahepatic vena cava or the hepatic veins) is almost completely transmitted back into the hepatic sinusoids. In addition, the increase in pressure is associated with an increase in filtration coefficient in the sinusoids and, therefore, in the permeability to proteins (1,32,33). In contrast, only 60% of the acute increase in portal venous pressure is transmitted back to the capillary bed of the small and large intestines and is associated with a decreased filtration coefficient. These effects represent a myogenic constriction of the arteriolar resistance and precapillary sphincters, which reduces microvascular pressure and the number of perfused capillaries. Second, the compliance (relation between interstitial pressure and interstitial volume) is much lower in the liver than in the intestine. Finally, the intestines, but not the liver, have an efficient lymphatic system for removing interstitial edema (1,32,33).

These differences between the hepatic and splanchnic microcirculation explain the findings observed in experimental animals after constriction of the suprahepatic vena cava or hepatic veins and partial ligation of the portal vein. Elevation of hepatic venous pressure is associated with a dramatic increase in the passage of fluid, with a protein concentration similar to that in the plasma from the sinusoidal lumen to the space of Disse. The macroscopic consequence of this is a marked enlargement of the liver. Because the compliance of the liver is low and the ability of the lymphatic system to remove the interstitial fluid insufficient, a marked increase in interstitial pressure ensues. This leads to leakage of hepatic lymph with very high protein concentration from the liver surface into the peritoneal cavity. This sequence of events probably occurs in Budd-Chiari syndrome and other clinical forms of suprahepatic portal hypertension, in which there is hepatomegaly as well as protein-rich ascites formation.

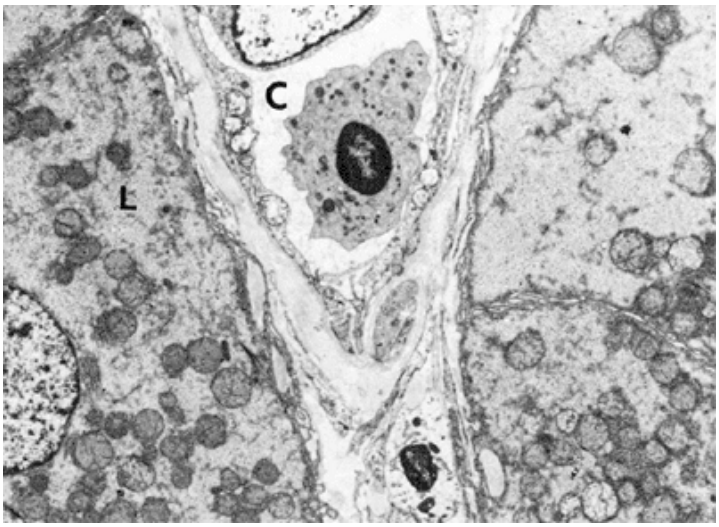
The elevation in portal venous pressure (e.g., after partial ligation of the portal vein) increases the formation of lymph with low protein concentration from the stomach, small intestine, and colon and is associated with local edema in these organs. However, there is no leakage of fluid into the abdominal cavity probably because of two factors. First, the acute increase in filtration is rapidly counterbalanced by an increase in the oncotic pressure difference between the capillary lumen and interstitial space, which limits the exit of fluid from the intravascular compartment. Second, the splanchnic lymphatic system is able to return most of the excess of lymph produced in the stomach and intestines to systemic circulation. Interestingly enough, and contrary to the process occurring in the normal liver in which an increase in hepatic venous pressure is associated with an increase in the lymph to plasma protein ratio to almost 1, in the intestine the increase in portal pressure decreases the lymph to plasma protein ratio to 0.20 (34,35).

Transvascular exchange of fluid and source of ascites in cirrhosis

The hepatic and the splanchnic transvascular exchange of fluid in cirrhosis differs

considerably from that in postsinusoidal and prehepatic portal hypertension. This is due to anatomic and functional changes occurring in the hepatic and splanchnic microcirculation during the course of cirrhosis. In the hepatic microcirculation there is a "capillarization" of the sinusoids, which means that the normal sinusoids become microvessels with continuous endothelial lining, lacking fenestra and supported by a basement membrane and collagenous tissue (33) (Fig. 19.4). This capillary-like structure and the sinusoidal structures are encountered in sequence in the same vascular pathway, the

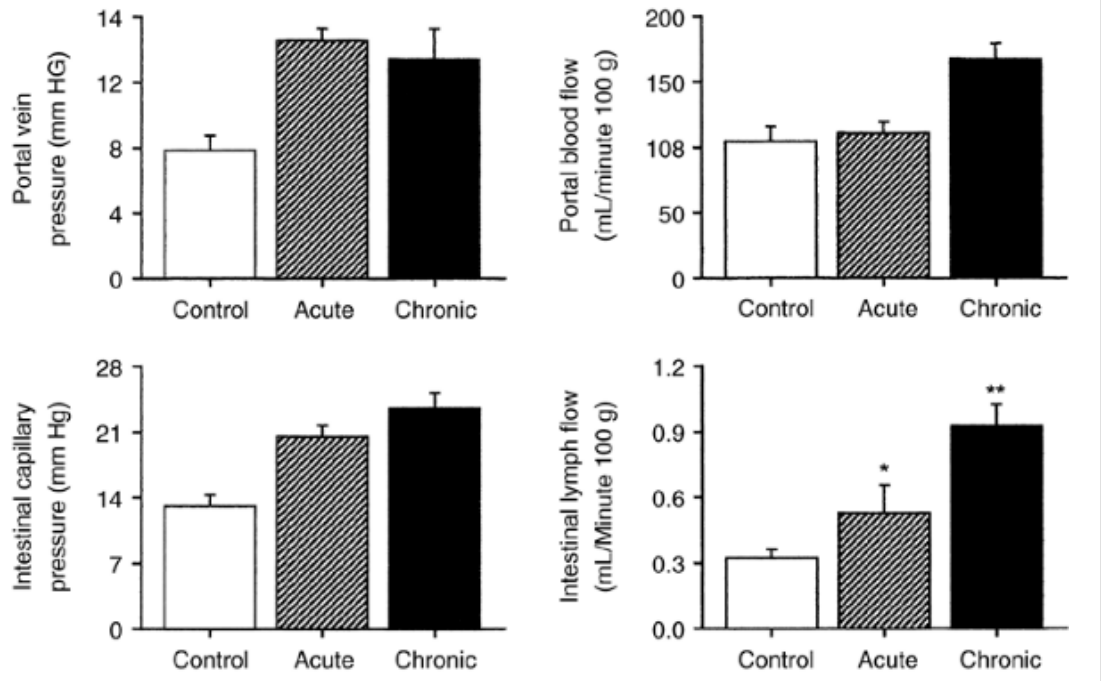
former being more commonly found at the periphery of the regenerative nodules. The degree of capillarization of the hepatic sinusoids, and, therefore, the permeability to albumin in the hepatic microcirculation, varies greatly from patient to patient. For example, whereas the volume of distribution of albumin in the normal liver exceeds that of red blood cells by 60% because of the passage of albumin but not cells into the space of Disse, in cirrhotic livers it may be as low as 5%, indicating a microcirculation almost totally impermeable to albumin (33). In some cirrhotic livers, however, this percentage may approach that found in normal livers. Not surprisingly, the hepatic lymph to plasma ratio for total proteins in cirrhosis ranges between 0.07 and 0.60 (mean value 0.50).



▪ **Figure 19.4** Capillarization of sinusoids in hepatic cirrhosis. An electron micrograph in which a capillary is seen between regenerative liver parenchymal cells. The capillary lumen (*C*) is separated from the liver cells (*L*) by the nonfenestrated endothelial cell, a basement membrane, and a layer of fibrillary collagen. (From Huet P-M, Goresky CA, Villeneuve JP, et al. Assessment of liver microcirculation in human cirrhosis. *J Clin Invest* 1982;70:1234–1244, with permission.)

In cirrhotic portal hypertension there is no autoregulation of capillary pressure and filtration coefficient in the splanchnic microcirculation. Instead of inducing a splanchnic vasoconstriction, portal hypertension in cirrhosis is associated with generalized splanchnic arterial vasodilatation (32). The increase in hydrostatic pressure in the splanchnic capillaries in cirrhosis is due to both a "backward" transmission of the increased portal pressure into the splanchnic microcirculation and a "forward" transmission of the high pressure in the arterial vascular

compartment to the splanchnic capillaries due to the decreased arterial vascular resistance (1). Results of experimental studies indicate that by far the most important mechanism of the increased hydrostatic pressure in the splanchnic microcirculation in portal hypertension is the reduction in arterial vascular resistance and, consequently, the increased inflow of blood at high pressure to this compartment (Fig. 19.5). This increase in hydrostatic pressure leads to a fall in lymph to plasma ratio of protein (0.20 vs. 0.50 to 0.60 in normal conditions). Not surprisingly, interstitial edema in the intestinal mucosal, muscular, and serosal layers is prominent in cirrhosis among humans.



▪ **Figure 19.5** Portal vein pressure, portal blood flow, intestinal capillary pressure and intestinal lymph flow in control and in acute and chronic portal hypertensive rats. *, $P < 0.05$ versus control; **, $P < 0.01$ versus 0.04. (From Korthuis RJ, Kinden DA, Brimer GE, et al. Intestinal capillary filtration in acute and chronic portal hypertension, *Am J Physiol* 1988;254:G339–G345, with permission.)

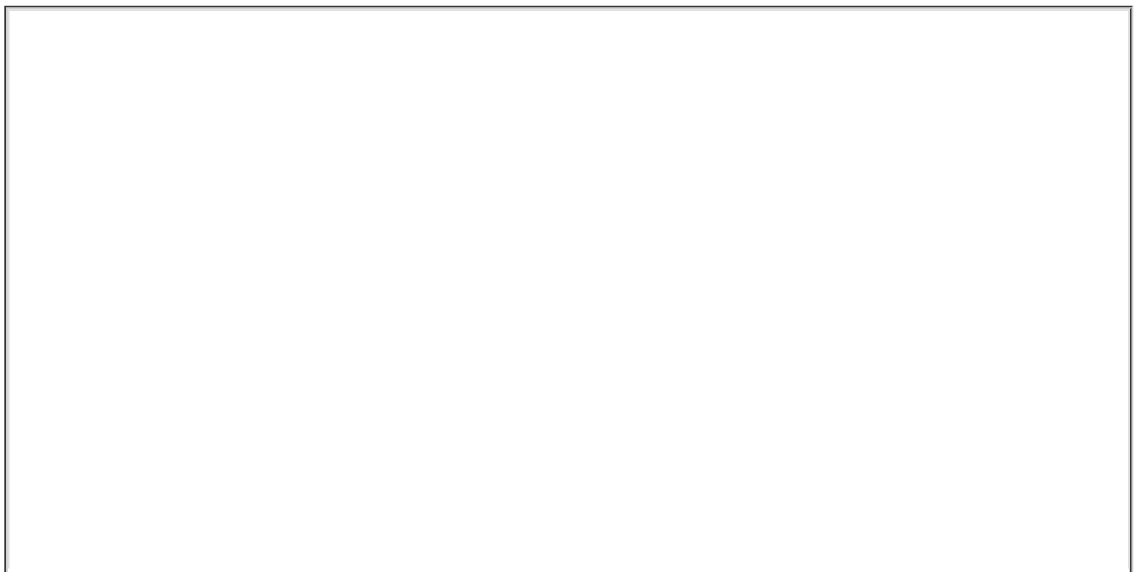
Vessels leaving the liver by the hilum principally drain liver lymph. Liver lymph, as well as that derived from other intra-abdominal organs (e.g., pancreas, spleen, stomach, large and small intestines and mesentery), drains into the thoracic duct. The thoracic duct is a 35- to 45-cm long lymphatic channel that begins in the upper lumbar region, passes through the diaphragm, ascends in the posterior mediastinum, and drains into the left subclavian or internal jugular vein. In healthy humans, thoracic duct lymph flow is approximately 1L/day. In cirrhosis it averages 8 to 9 L/day, and may be higher than 20 L/day, indicating a high filtration of fluid from the hepatic and splanchnic intravascular compartment into the interstitial space (35). There is evidence that the lymphatic system is efficient in returning most of this fluid into the intravascular compartment in patients with cirrhosis and ascites. Less than 5% of the albumin leaving the intravascular compartment (an estimation of the dynamic of fluid through the

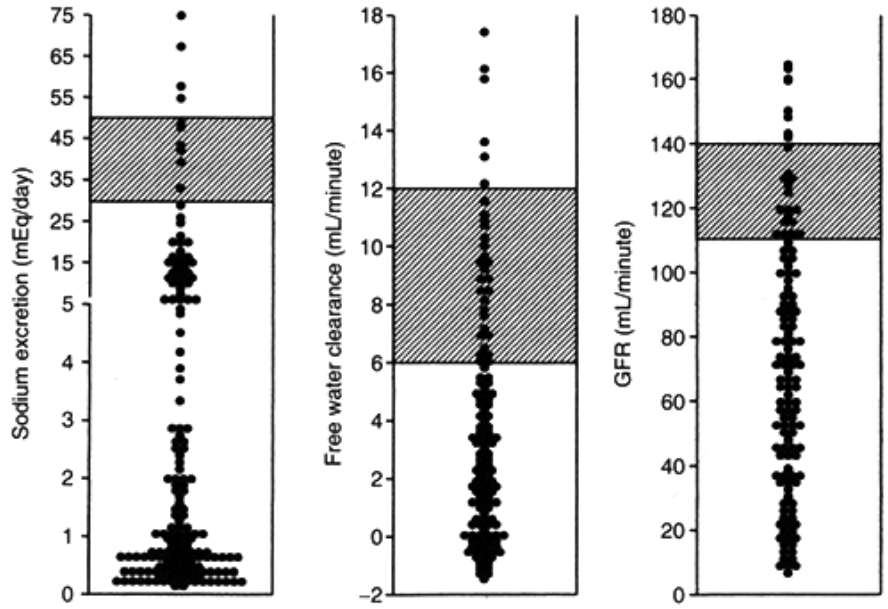
sinusoids and splanchnic capillaries) escapes into the peritoneal cavity (34). Ascites formation in cirrhosis is therefore the consequence of a small spillover of the increased hepatic and splanchnic lymph formation, most of which is returned directly to the circulation through the lymphatic system.

The source of ascites in cirrhosis has never been specifically investigated. The traditional concept that ascites in cirrhosis is derived mainly from the hepatic vascular compartment is not in agreement with the data presented earlier. The total concentration of protein in ascitic fluid and thoracic duct lymph in most patients with cirrhosis is lower than that in the hepatic lymph. This finding indicates a significant contribution of the splanchnic organs to the formation of ascites. In patients with advanced decompensated cirrhosis, severe portal hypertension, intense splanchnic arterial vasodilatation, and very low total protein concentration in ascitic fluid, most cases of ascites are probably the result of intestinal (and filtration by other splanchnic organs) filtration.

Reabsorption of ascitic fluid

The volume of ascites depends not only on the amount of hepatic and splanchnic interstitial fluid leaking into the peritoneal cavity but also on the rate of reabsorption of ascitic fluid into the intravascular compartment. The lymphatic vessels on the undersurface of the diaphragm play an important role in this latter process. These vessels and the diaphragmatic peritoneum are especially prepared for this function. A single layer of mesothelial cells covers the peritoneal surface of the diaphragm over a connective tissue matrix with a very rich plexus of terminal lymphatic vessels (lymphatic lacunae) (36,37,38). The submesothelial connective tissue over the lymphatic lacunae is almost absent and wide gaps, large enough to allow the passage of erythrocytes, connect the peritoneal cavity with the lumen of the terminal lymphatics. The submesothelial lymphatic plexus drains into a deeper plexus of valved collecting vessels, which penetrates connecting tissue septa between the muscular fibers of the diaphragm and drain into parasternal trunks on the ventral thoracic wall, right lymphatic duct, and right subclavian or internal jugular vein. During inspiration, intercellular gaps close, intraperitoneal pressure increases, and the lacunae are emptied through the combined effects of local compression, increased intra-abdominal pressure, and reduced intrathoracic pressure. During expiration, the gaps open and free communication is reestablished.





▪ **Figure 19.6** Sodium excretion, free water clearance, and glomerular filtration rate (GFR) in a large series of patients with cirrhosis and ascites. Shadowed areas indicate normal values in healthy subjects. Measurements of sodium excretion in healthy subjects and patients with cirrhosis were done under conditions of sodium restriction (50 mEq/day). Free water clearance was measured after an intravenous water load of 20 mg/kg body weight (5% dextrose solution).

Reabsorption of ascites in cirrhosis is a rate-limited process. The estimated mean rate of ascitic fluid reabsorption is 1.4 L/day, ranging from less than 0.5L to more than 4L. The low rates of ascites formation and reabsorption do not mean that the intraperitoneal cavity is almost isolated from the rest of the body. The transperitoneal exchange of water and water-soluble substances (e.g., antibiotics not bound to proteins) by diffusion is rapid in patients with cirrhosis and ascites.

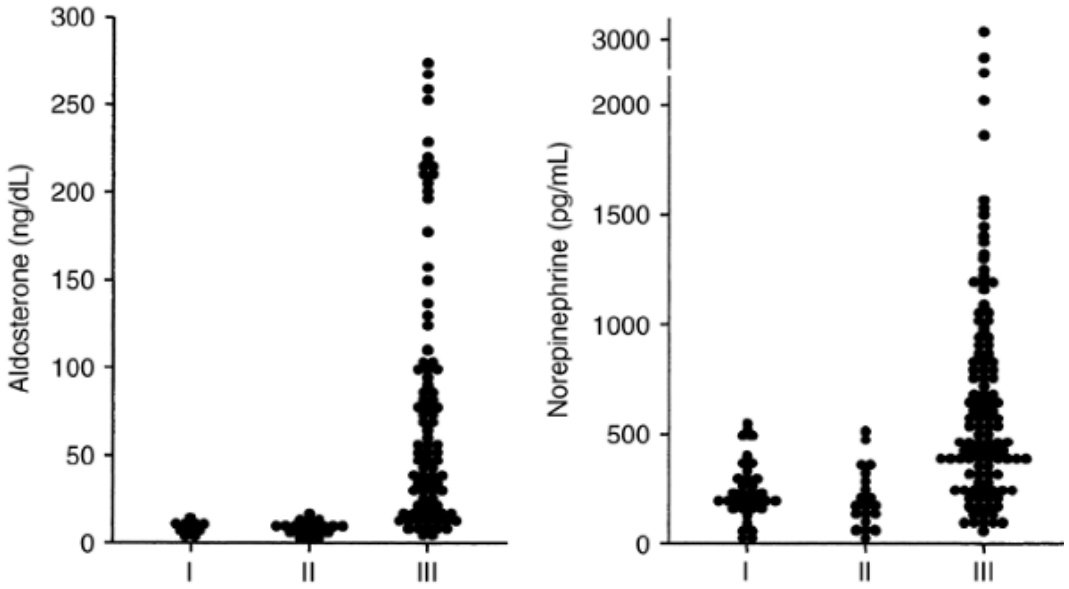
Renal and Circulatory Dysfunction: Role in the Formation of Ascites

Renal dysfunction in cirrhosis

Renal sodium retention, as well as the secondary retention of water, is the second important factor in the formation of ascites (Fig. 19.6). The mechanism of

this abnormality is multifactorial. The renin–angiotensin–aldosterone system, which stimulates sodium reabsorption in the distal nephron, and the sympathetic nervous system, which increases sodium reabsorption in the proximal tubule, loop of Henle, and distal tubule, are stimulated in a significant number of patients with cirrhosis and ascites but not in those with compensated cirrhosis (Fig. 19.7). Glomerular filtration rate (GFR) is markedly reduced in some patients with decompensated cirrhosis and may contribute to sodium retention. However, 30% of patients with cirrhosis, sodium retention, and ascites show plasma concentration of aldosterone and norepinephrine (a sensitive marker of the

sympathetic nervous activity) and GFR within normal limits. This finding indicates that other, still unknown mechanisms participate in the pathogenesis of sodium retention in cirrhosis. The circulating plasma levels of natriuretic peptides (i.e., atrial natriuretic peptide, brain natriuretic peptide) (39) are markedly increased in patients with decompensated cirrhosis. Therefore, sodium retention occurs despite an increased synthesis of these endogenous natriuretic hormones.



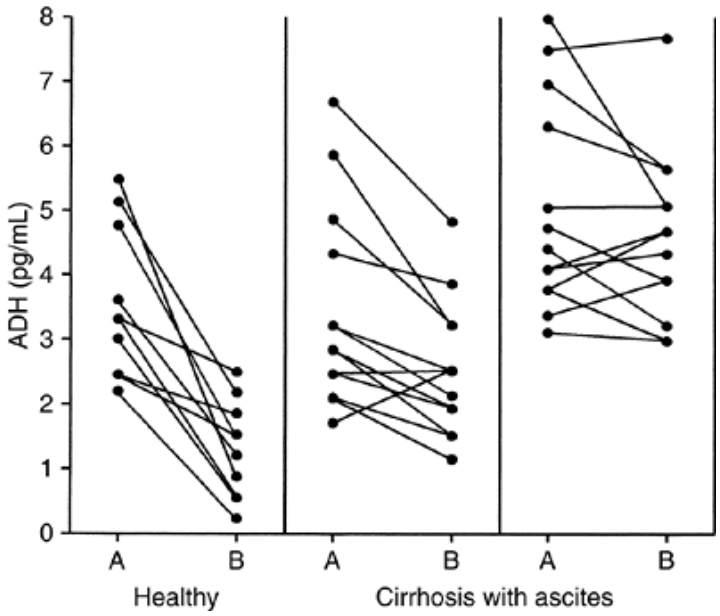
▪ **Figure 19.7** Aldosterone and norepinephrine levels in healthy subjects (*I*), compensated patients with cirrhosis (*II*), and patients with cirrhosis and ascites (*III*). (From Arroyo V, Planas R, Gaya J, et al. Sympathetic nervous activity, renin-angiotensin system and excretion of prostaglandin E2 in cirrhosis. Relationship to functional renal failure and sodium and water excretion. *Eur J Clin Invest* 1983;13;271–278, with permission.)

As decompensated liver diseases progresses, patients develop a decreased renal ability to excrete free water. When this function is severely depressed, patients become unable to excrete the excess of water ingested with the diet. This water dilutes the interior milieu and produces hyponatremia and hypo-osmolality. Water retention and dilutional hyponatremia develop months after the onset of sodium retention and ascites and are secondary to a nonosmotic hypersecretion of antidiuretic hormone (Fig. 19.8). Water retention in patients with dilutional hyponatremia is a part of the positive fluid balance and contributes to the formation of ascites.

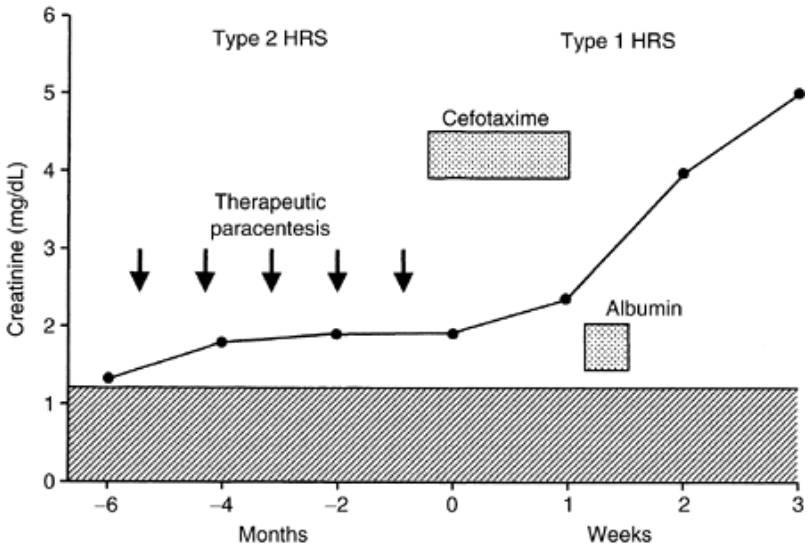
At the terminal stage of the disease patients have HRS (4). This is a functional renal failure due to an intense vasoconstriction of the renal arteries, which causes a decrease in renal perfusion and GFR. Two types of HRS have been identified (Fig. 19.9). Type 2 HRS involves a moderate renal failure (serum creatinine between 1.5 and 2.5 mg/dL; upper normal level 1.2 mg/dL) that

remains steady during relatively long periods (months). Type 1 HRS is a rapidly progressive renal failure. It usually develops in patients who already have type 2 HRS in close chronologic relation to a precipitating event, such as bacterial

infection, gastrointestinal hemorrhage, or major surgical procedure. In type 1 HRS, the serum creatinine level can become very high (>4 to 5 mg/dL) in a short period (days or weeks). The prognosis of patients with type 1 HRS is extremely poor (80% of patients die within 1 month of the onset of the syndrome).



▪ **Figure 19.8** Antidiuretic hormone (ADH) levels in healthy subjects and in patients with cirrhosis and ascites after water restriction (*A*) and after water loading (*B*). Patients with cirrhosis and ascites are divided into two groups: Those with positive free water clearance after a water load (20 mL/kg of body weight) (*middle graph*) and those with negative free water clearance and dilutional hyponatremia. There was an inverse relationship between free water clearance and ADH. (From Pérez-Ayuso RM, Arroyo V, Camps J, et al. Evidence that renal prostaglandins are involved in renal water metabolism in cirrhosis. *Kidney Int* 1984;26:72–80, with permission.)



▪ **Figure 19.9** A typical patient with type 2 hepatorenal syndrome (HRS) and refractory ascites who developed type 1 HRS in close chronologic relationship with spontaneous bacterial peritonitis. Despite the rapid resolution of the infection, the patient developed a rapidly progressive renal failure and died.

Circulatory dysfunction and peripheral arterial vasodilatation in cirrhosis: Relationship with renal dysfunction and intrahepatic hemodynamics

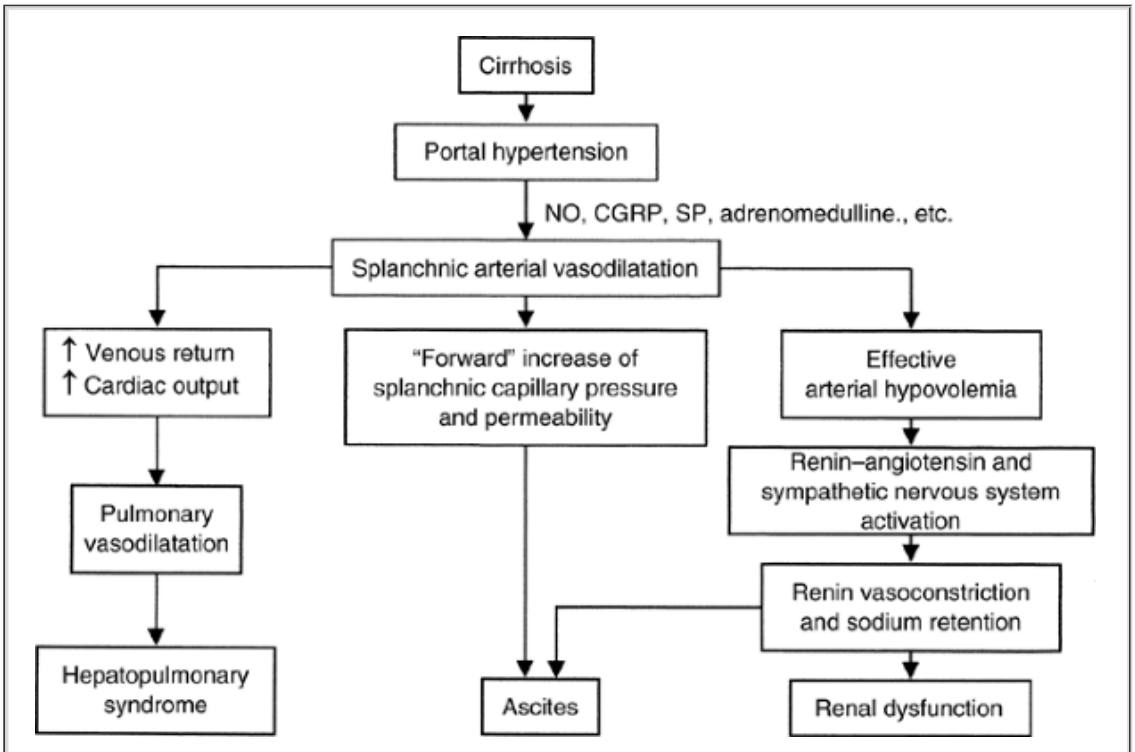
Renal dysfunction in patients with cirrhosis occurs in the setting of a circulatory dysfunction characterized by a marked arterial vasodilatation (40,41). There is evidence that the splanchnic circulation is the site of this arterial vasodilatation because there is vasoconstriction in all the other major vascular territories such as the kidneys, muscle, skin, and brain (42,43,44). In contrast, in the splanchnic circulation there is vasodilatation, which increases the inflow of blood into the portal venous system (26). Indirect evidence suggests that the degree of splanchnic arterial vasodilatation in cirrhosis with ascites is intense because the hepatic blood flow is normal although 60% to 80% of the portal flow is shunted through collateral circulation. The splanchnic arterial blood flow in cirrhosis, therefore, may be double that in healthy subjects. The splanchnic circulation is also the predominant site where arterial vasodilatation occurs in patients with compensated cirrhosis.

It is well established that splanchnic arterial vasodilatation in cirrhosis is related to portal hypertension. It plays a major role in the maintenance of increased portal pressure despite the development of collateral circulation. The mechanism by which increased portal pressure decreases splanchnic arterial vascular resistance is not well understood. For many years arterial vasodilatation in cirrhosis has been attributed to increased circulating plasma levels of vasodilators such as glucagon, prostaglandins, adrenomedullin, and natriuretic peptide. However, because the site of arterial vasodilatation is the splanchnic circulation, a local mechanism (increased release of a vasodilator substance within the splanchnic area) is a more likely hypothesis. Results of recent studies suggesting that nitric oxide, a vasodilator substance that acts in a paracrine manner, is important in the pathogenesis of splanchnic arterial vasodilatation in cirrhosis is consistent with this hypothesis. Increased activity of nitric oxide synthase in the splanchnic circulation has been reported in experimental cirrhosis (45,46,47). On the other hand, inhibition of nitric oxide normalizes circulatory function in experimental cirrhosis (47). Two hypotheses have been proposed to explain the mechanism of the increased production of nitric oxide in the splanchnic circulation. The first is that it occurs secondary to bacterial translocation from the intestinal lumen to the interstitial intestinal space. Endotoxin and the increased cytokine production stimulate the activity of nitric oxide synthase in the endothelial and vascular smooth muscle cells (46,48). The second hypothesis

considers that there is a stimulation of the nonadrenergic, noncholinergic nervous system secondary to portal hypertension (46,49). This is a sensitive system that, when activated, releases numerous vasodilatory neurotransmitters, including nitric oxide, calcitonin gene–related peptide, substance P, and vasoactive intestinal peptide (50,51). Nonadrenergic, noncholinergic terminals are abundant

not only in the gastrointestinal smooth muscle but also in the vascular smooth muscle cells. It may be possible that portal hypertension induces changes in the intestinal wall (increase in interstitial pressure and interstitial edema) that stimulate this system and cause an inhibitory effect on the gastrointestinal smooth muscle cells. The gastrointestinal transit time is greatly prolonged in patients with cirrhosis; this finding indicates inhibition of gastrointestinal motility (52).

The circulatory dysfunction induced by the splanchnic arterial vasodilatation is the primary mechanism implicated in the pathogenesis of complications in patients with cirrhosis. The impairment of renal function is the most characteristic complication induced by the circulatory dysfunction (Fig. 19.10). It is also the mechanism of hepatopulmonary syndrome, which is characterized by mild to severe hypoxemia in the absence of associated cardiopulmonary disease. The hypoxemia is caused by vasodilatation in the intrapulmonary circulation. Finally, although the distortion of the liver vascular architecture caused by fibrosis and nodule formation is the most important mechanism of the increased intrahepatic vascular resistance in cirrhosis, there is a functional component of portal hypertension because of an increase in the intrahepatic vascular tone. The contractile intrahepatic vascular elements include the vascular smooth muscle cells from the small venules and the hepatic stellate cells that surround the sinusoids. In cirrhosis these stellate cells undergo a phenotypic transformation, acquiring receptors for numerous endogenous vasoactive substances, including angiotensin II, norepinephrine, antidiuretic hormone, and endothelin, and contractile properties. Therefore, the circulatory dysfunction in cirrhosis and the secondary activation of these endogenous vasoactive substances may affect the functional component of the intrahepatic resistance to the portal venous flow and increase portal pressure.

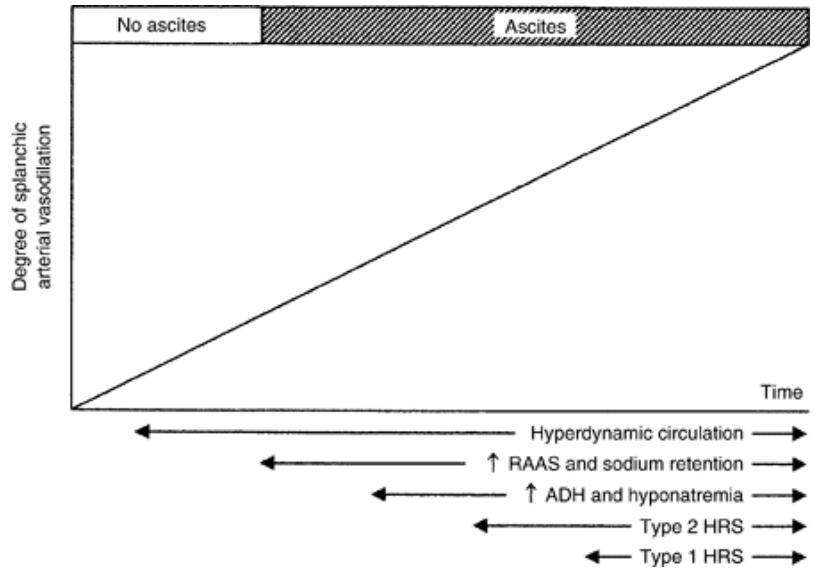


▪ **Figure 19.10** Peripheral arterial vasodilatation hypothesis. NO, nitric

oxide; CGRP, calcitonin gene–related peptide; SP, substance P.

In the initial stages of cirrhosis, circulatory dysfunction is compensated by a hyperdynamic circulation (Fig. 19.11). Plasma volume, cardiac output, and heart rate increase and the circulatory transit time decreases. The splanchnic circulation behaves functionally as an arteriovenous fistula. The incidence of arterial hypertension in patients with cirrhosis and portal hypertension is very low because of this circulatory abnormality. With the progression of the liver disease and the accentuation of portal hypertension and splanchnic arterial vasodilatation, patients develop sodium retention and ascites. In the initial phases of ascites, the renin–angiotensin and the sympathetic nervous systems are not stimulated, and the mechanism of sodium retention in this period is unknown. Later during the course of the disease, the renin–angiotensin–aldosterone system and the sympathetic nervous system become progressively activated in parallel with more intense reduction in urine sodium excretion. Patients with ascites and normal plasma

renin activity and aldosterone concentration, in general, have urinary sodium excretion over 10 mEq/day, and they respond easily to low diuretic dosage. In contrast, most patients with high renin and aldosterone concentration show a urinary sodium excretion lower than 5 mEq/day (in many cases, almost zero) and need high diuretic dosage to achieve a natriuretic response. Hypersecretion of antidiuretic hormone occurs at later stages of the disease. This explains why hyponatremia is a late event in decompensated cirrhosis. This is probably related to the fact that antidiuretic hormone is less sensitive than the sympathetic nervous system and the renin–angiotensin system to changes in the effective circulating blood volume. HRS develops at the very late stages of the disease, always in the setting of an intense activation of the renin–angiotensin and sympathetic nervous systems and antidiuretic hormone.



▪ **Figure 19.11** Temporal relationship between the degree of splanchnic

arterial vasodilatation and the appearance of the various disorders of renal function in cirrhosis. RAAS, renin–angiotensin–aldosterone system; ADH, antidiuretic hormone; HRS, hepatorenal syndrome. (From Arroyo V, Jimenez W. Complications of cirrhosis II. Renal and circulatory dysfunction. Lights and shadows in an important clinical problem. *J Hepatol* 2000;32(suppl 1):157–170, with permission.)

The administration of specific antagonists of the vascular effect of angiotensin II or antidiuretic hormone (V1 antagonists) to experimental animals or patients with cirrhosis and ascites is associated with a profound hypotensive response secondary to a decrease in peripheral vascular resistance. This effect, which is not observed in healthy persons or in patients with compensated cirrhosis (patients who have never had ascites), indicates that activation of the renin–angiotensin system, sympathetic nervous system, and antidiuretic hormone in cirrhosis with ascites is a homeostatic response to maintain arterial pressure at normal or nearly normal levels. Arterial vasodilatation and the secondary arterial hypotension are therefore stimuli leading to the activation of these systems.

The different phases of cirrhosis in the development of ascites and abnormalities of renal function parallel the progression of portal hypertension and splanchnic arterial vasodilatation. In patients with cirrhosis there is a strong direct relationship between the degree of portal hypertension; plasma level of renin, aldosterone, and norepinephrine; and the intensity of sodium retention. Arterial pressure is lower in patients with cirrhosis and ascites than in those with compensated cirrhosis. Finally, among patients with ascites, those with HRS present with the lowest arterial pressure and the highest plasma levels of renin, norepinephrine, and antidiuretic hormone.

Renal and other extrasplanchnic regional circulations in cirrhosis

Traditional studies with para-aminohippurate clearance and recent investigations with echo-Doppler technique have shown increased intrarenal vascular resistance in patients with cirrhosis and ascites before the development of HRS. Therefore, HRS is the extreme expression of an impairment of renal circulatory function that starts at earlier stages. Renal plasma flow, intrarenal vascular resistance, and GFR in cirrhosis with ascites closely correlate with the degree of stimulation of the renin–angiotensin system and the

sympathetic nervous system (53). Patients with normal or moderately increased plasma levels of renin and norepinephrine usually show normal renal perfusion and GFR, whereas the levels of these substances are markedly increased in patients with HRS (Table 19.2). These data have led to the contention that HRS in cirrhosis is caused by renal vasoconstriction related to the activation of these systems (3). However, this hypothesis is too simple, and at present, there is evidence that intrarenal mechanisms may also participate in the regulation of renal perfusion.

Table 19.2. Mean Arterial Pressure, Plasma Volume, Cardiac Index, Plasma Renin Activity, and Norepinephrine Levels in Patients with

Cirrhosis with and without Hepatorenal Syndrome and in Healthy Subjects

		Cirrhosis with ascites	
	Healthy subjects	**No HRS**	**HRS**
MAP (mm Hg)	87 ± 3	82 ± 2	69 ± 5
Plasma volume (mL/kg)	44 ± 2	66 ± 2	59 ± 4
Cardiac index (L/min m^2)	3.0 ± 0.2	5.7 ± 0.2	5.5 ± 0.5
Plasma renin activity (ng/mL h)	0.5 ± 0.1	8.2 ± 2	31.7 ± 10.4
Norepinephrine (pg/mL)	200 ± 22	512 ± 39	1,141 ± 134

P < 0.001 for all values (analysis of variance [ANOVA]).
HRS, hepatorenal syndrome; MAP, mean arterial pressure.

The kidneys synthesize vasodilator substances, the most important of which are prostaglandins, particularly prostaglandin E_2, and prostacyclin. The renal synthesis of prostaglandins increases whenever there is an increased activity of the renin–angiotensin and sympathetic nervous systems (45). Prostaglandins antagonize the vasoconstrictor effect of angiotensin II and norepinephrine and, by this mechanism, play an essential role in the maintenance of renal perfusion and GFR in conditions such as decompensated cirrhosis or congestive heart failure, in which there is circulatory dysfunction. A syndrome similar to HRS can be produced in patients with nonazotemic cirrhosis and ascites by the administration of nonsteroidal anti-inflammatory drugs, which inhibit prostaglandin synthesis (53,54). Investigations in experimental animals with cirrhosis and ascites have shown that the renal production of nitric oxide also participates in the maintenance of renal perfusion (55). Finally, the administration of antagonists of the vascular receptors of natriuretic peptides in animals with cirrhosis and ascites induces an impairment of renal function that mimics HRS (56). Therefore, intrarenal and circulating vasodilatory substances contribute to the maintenance of renal perfusion in cirrhosis with ascites. HRS develops when the renal production of these substances is insufficient to antagonize the renal effects of the endogenous vasoconstrictor systems. This can occur when there is a stimulation of the vasoconstrictor systems, a reduction in the synthesis of vasodilators, or both.

Prostaglandin synthesis is initiated by the transformation of membrane

phospholipids to arachidonic acid, a process mediated by phospholipase A_2. Subsequently, arachidonic acid is converted into the endoperoxides prostaglandin G_2 and prostaglandin H_2 by the action of the enzyme cyclo-oxygenase (COX). Two types of COX exist, COX-1, which plays an important role in many physiologic processes including the protection of the gastric mucosa and the regulation of renal perfusion, and COX-2, which is involved in the inflammatory process. Aspirin and the traditional nonsteroidal anti-inflammatory drugs (e.g., indomethacin, ibuprofen, and diclofenac) inhibit both COX-1 and COX-2. Therefore, in addition to their anti-inflammatory effect, they may produce lesions in the gastric mucosa and, in edematous patients, renal failure. Recently, specific inhibitors of COX-2 have been developed. Studies in experimental animals with cirrhosis and ascites and a recent investigation in patients with cirrhosis and ascites who have an increased activity of the renin–angiotensin system suggest that COX-2 inhibitors do not impair renal function in decompensated cirrhosis (57).

The kidney produces vasoconstrictor substances, such as angiotensin II, endothelin, and adenosine. The production of these substances is stimulated in conditions of renal hypoperfusion. Therefore, these substances could also participate in the pathogenesis of HRS. If fact, it has been proposed that when severe renal hypoperfusion develops in cirrhosis with ascites, there could be a reduction of the intrarenal synthesis of vasodilators and a stimulation of the renal synthesis of vasoconstrictors secondary to renal ischemia, thereby creating vicious circles that lead to a rapidly progressive impairment of renal perfusion and GFR (type 1 HRS). This could explain why type 1 HRS usually occurs in patients with type 2 HRS, who already have a precarious equilibrium between vasoconstrictor and vasodilator mechanisms, and after a precipitating event (e.g., paracentesis, hemorrhage, and bacterial infection) that produces a further deterioration in circulatory function and renal perfusion. Once type 1 HRS develops, it progresses by the intrarenal mechanisms, independent of the correction of the precipitating event. Only the normalization of circulatory function can reverse these intrarenal vicious circles and improve renal perfusion and GFR in patients with type 1 HRS.

Doppler studies of the brachial and femoral arteries, which supply blood mainly to the skin and muscles, and the middle cerebral artery, which supplies

approximately 75% of the blood in the cerebral hemispheres, in patients with cirrhosis and ascites have also shown the presence of vasoconstriction in these vascular territories (42,43,44). Because cutaneous, muscular, and cerebral vascular resistance in patients with cirrhosis and ascites parallels renal vascular resistance and correlates closely with the degree of activity of the renin–angiotensin and the sympathetic nervous systems, it is clear that changes in these regional circulations in decompensated cirrhosis represent a homeostatic response to maintain the arterial pressure.

An important point is that splanchnic arterial vasodilatation persists in decompensated cirrhosis despite the marked stimulation of the renin–angiotensin and the sympathetic nervous systems and the nonosmotic hypersecretion of antidiuretic hormone. This phenomenon is caused by marked resistance of the splanchnic arterioles to the vasoconstrictor effect of angiotensin II, noradrenaline, and vasopressin. Data on experimental cirrhosis suggest the resistance is caused by increased local synthesis of nitric oxide because inhibition of nitric oxide synthase normalizes the response of the splanchnic circulation to

these vasoconstrictors. Therefore, splanchnic arterial vasodilatation in cirrhosis progresses with the increase in portal hypertension, induces the activation of the endogenous vasoconstrictor systems, and leads to vasoconstriction in the extrasplanchnic vascular territories. Because the splanchnic circulation in cirrhosis has little capacity to participate in the homeostasis of arterial pressure owing to the lack of response to vasoconstrictors, muscular and cutaneous blood flow is very low under rest conditions, and the cerebral circulation is regulated by effective mechanisms, the maintenance of circulatory function in cirrhosis relies mainly on the renal circulation. This explains why patients with cirrhosis and ascites are highly prone to the development of renal impairment and HRS in conditions associated with an impairment of circulatory function, such as bacterial infections, paracentesis, hemorrhage, and diuretic treatment.

Cardiac dysfunction in cirrhosis: A second important mechanism of circulatory and renal dysfunction and ascites

Research on circulatory function in cirrhosis has focused for many years on the peripheral arterial circulation. Recent studies, however, suggest that in cirrhosis cardiac dysfunction is also present, which could be of major importance in the deterioration of circulatory and renal function and the pathogenesis of ascites and HRS (3,5). As indicated previously, arterial vasodilatation in the splanchnic circulation increases during the course of the disease, leading to homeostatic activation of the renin–angiotensin and sympathetic nervous systems to maintain arterial pressure. This progressive decrease in cardiac afterload should be followed by an increase in cardiac output and heart rate. However, this is not the case (Table 19.3). Heart rate in patients with nonazotemic cirrhosis, ascites, and normal or slightly increased activity of the renin–angiotensin and sympathetic nervous systems is similar to that in nonazotemic patients with increased activity of these systems or with HRS, indicating a severe impairment of cardiac chronotropic function. On the other hand, the cardiac output, although higher than normal in most cases, decreases progressively during the course of the disease. The mechanisms of circulatory dysfunction in cirrhosis may be, therefore, more complex than that proposed by the peripheral arterial vasodilatation hypothesis (Fig. 19.12). In patients with compensated cirrhosis, the splanchnic arterial vasodilatation is compensated by an appropriated cardiac response, with increased heart rate, left ventricular systolic ejection fraction, and cardiac output. However, with the progression of liver failure and portal hypertension this compensatory mechanism fails. The increase in arterial vasodilatation is not followed by an increase in heart rate. On the other hand, the cardiac output decreases rather than increases. Arterial pressure homeostasis is, therefore, solely dependent on the stimulation of the endogenous vasoconstrictor systems (i.e., renin–angiotensin system, sympathetic nervous system, and antidiuretic hormone), which has deleterious effects on renal perfusion and on the perfusion of other organs and produces sodium retention and leads to ascites formation.

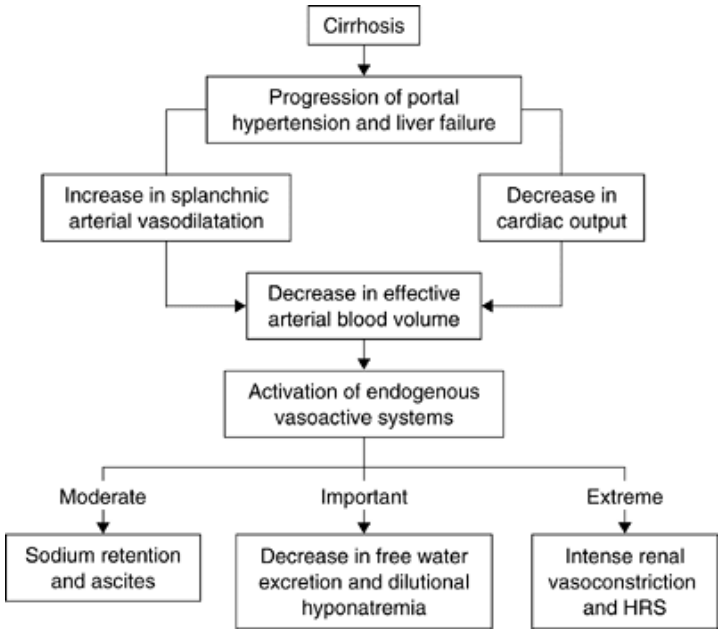
Table 19.3. Chronologic Changes of Vasoactive Systems and Cardiovascular Function from Nonazotemic Cirrhosis with Ascites to Hepatorenal Syndrome

	NA-1	NA-2	HRS
MAP (mm Hg)	88 ± 9	83 ± 9	75 ± 7
PRA (mg/mL h)	3 ± 2	10 ± 5	17 ± 14
NE (pg/mL)	221 ± 68	571 ± 241	965 ± 502
SVR (dyne s/cm^5)	962 ± 256	1,058 ± 265	1,096 ± 327
CO (L/min)	7.2 ± 1.8	6.0 ± 1.2	5.4 ± 1.5
HR (beats/min)	87 ± 15	85 ± 13	82 ± 14

Changes in plasma renin activity, norepinephrine concentration, and cardiac output were statistically significant ($P < 0.01$).
NA, patients with nonazotemic cirrhosis and ascites; NA-1, patients with normal or slightly increased plasma renin activity and norepinephrine concentration; NA-2, patients with high plasma renin activity and norepinephrine concentration; HRS, hepatorenal syndrome; MAP, mean arterial pressure; PRA, plasma renin activity; NE, norepinephrine concentration; SVR, systemic vascular resistance; CO, cardiac output; HR, heart rate.
From Ruiz del Arbol L, Urman J, Fernández J, et al. Systemic, renal and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003;38:1210–1218, with permission.

Cardiac chronotropic dysfunction in cirrhosis is probably related to the downregulation of β-adrenergic

receptors owing to the overactivity of the sympathetic nervous system. The decrease in cardiac output is probably related to a reduction in cardiac preload (3). There is a cirrhotic cardiomyopathy characterized by an impaired left ventricular diastolic function and cardiac hypertrophy (58,59). It is, however, unlikely that it plays a significant role in the decrease in cardiac function because in decompensated cirrhosis cardiac output increases after maneuvers that expand the central blood volume (e.g., head-out water immersion, plasma volume expansion, therapeutic paracentesis, and insertion of a peritoneovenous or a TIPS), indicating a preserved cardiac reserve. Cardiac dysfunction in cirrhosis, therefore, appears to be a functional disorder unrelated to the structural changes in the heart.



▪ **Figure 19.12** The new hypothesis of circulatory dysfunction in cirrhosis. HRS, hepatorenal syndrome.

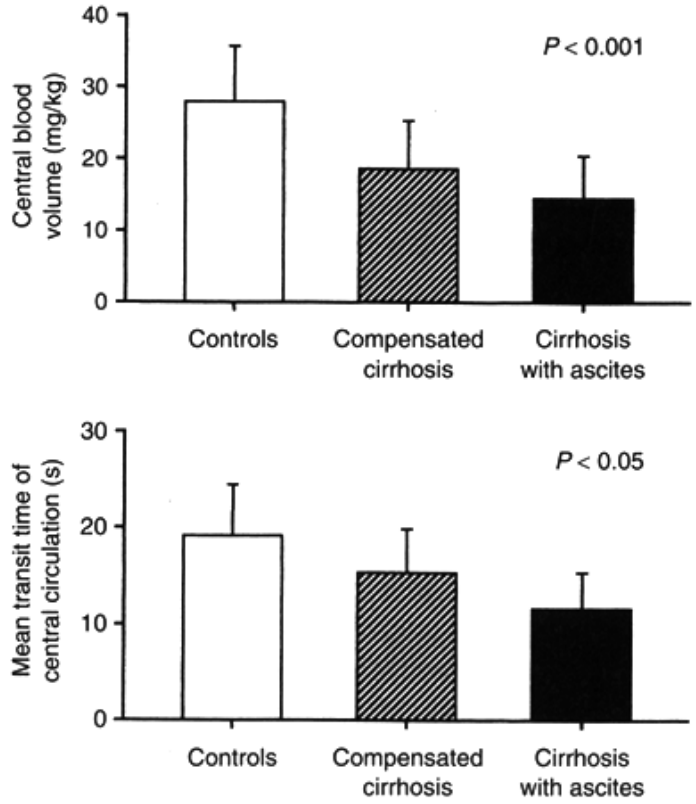
Pathogenesis of Ascites in Cirrhosis: The Forward Theory of Ascites

The previous discussion shows that our concept on ascites formation is moving from the portal venous system to the splanchnic arterial vascular compartment. Ascites formation in cirrhosis was traditionally considered to be due to a rupture of the Starling equilibrium within the splanchnic microcirculation secondary to a backward transmission of the increased intrahepatic and portal pressure to the sinusoids and splanchnic capillaries, respectively, and to hypoalbuminemia. According to this traditional theory, renal dysfunction is the consequence of a reduction in circulating blood volume secondary to the leakage of intravascular fluid to the peritoneal cavity. The fact that plasma volume and cardiac output are not reduced, but rather increased, in most patients with cirrhosis and ascites, however, invalidates this hypothesis. The low peripheral vascular resistance in decompensated cirrhosis is also evidence against this theory because circulating hypovolemia is associated with arterial vasoconstriction rather than with arterial vasodilatation.

The concept of effective hypovolemia was later proposed. Although circulating blood volume is increased in cirrhosis with ascites, the effective blood volume (the fraction of the blood volume present at a particular instant within the intrathoracic circulation that is able to influence low-pressure and high-pressure baroreceptors and, therefore, the sympathetic nervous activity, the renin–angiotensin system, and antidiuretic hormone) is actually reduced. This promotes sodium and water retention, which contributes to the formation of ascites. Results of subsequent studies confirmed this hypothesis. The transit time of blood within the intrathoracic vascular compartment is very short in patients with cirrhosis and ascites because of extremely rapid circulation as a consequence of the arterial vasodilatation. On the other hand, the intrathoracic blood volume is

reduced in patients with cirrhosis and ascites compared with that in patients with compensated cirrhosis and in healthy persons (Fig. 19.13). Therefore, although the blood volume circulating per unit time (i.e., per minute) throughout the intrathoracic vascular compartment is increased in patients with ascites, the intrathoracic blood volume present at a particular moment

is reduced owing to the hyperdynamic circulation. The splanchnic circulation, therefore, behaves as an arteriovenous fistula in decompensated cirrhosis (60). A large volume of blood enters into and leaves the portal venous system rapidly owing to the reduced splanchnic vascular resistance and the existence of portocollateral circulation. The hyperdynamic circulation leads to a vasodilatation in the pulmonary circulation to allocate the increased venous return, and this effect may be associated with an abnormal ventilation/perfusion ratio and low arterial oxygen saturation. Finally, increased venous return and arterial hypotension in the systemic circulation lead to increased stroke volume, tachycardia and, consequently, increased cardiac output. This closes the circle of the hyperdynamic circulation in decompensated cirrhosis.



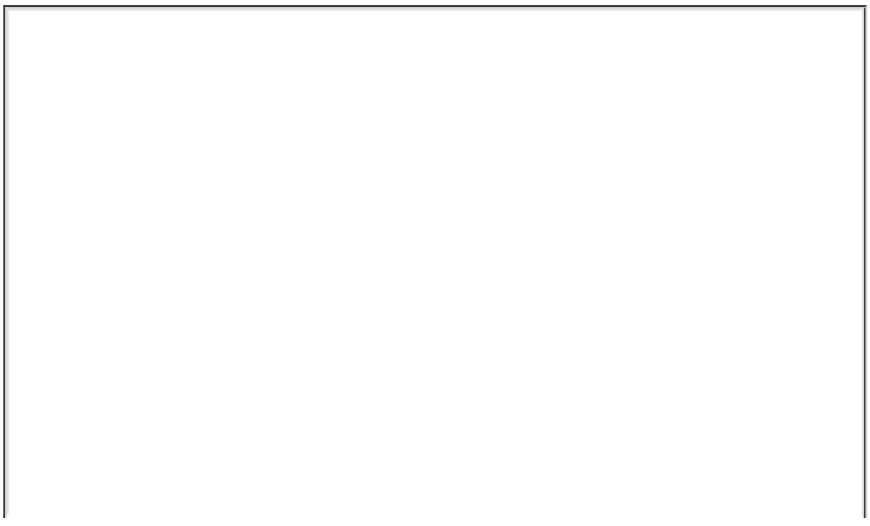
▪ **Figure 19.13** Central blood volume and mean transit time of central circulation in controls, in compensated cirrhosis, and in cirrhosis with ascites. (From Henriksen JH, Bendstsen F, Sorensen TI, et al. Reduced central blood volume in cirrhosis, *Gastroenterology* 1989;97:1506–1513, with permission.)

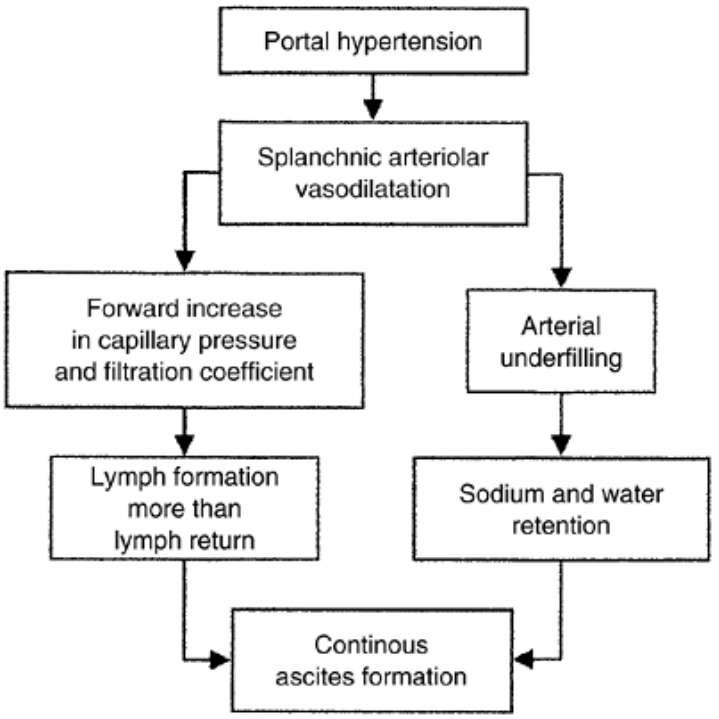
The recent demonstration that the hyperdynamic circulation diminishes during the course of the disease because of a decrease in the cardiac output adds a new

dimension to the pathogenesis of circulatory dysfunction and ascites formation in cirrhosis (3). As the disease progresses, the hyperdynamic circulation, which is intense before and soon after the development of ascites, decreases, contributing to the stimulation of the endogenous vasoconstrictor systems. Angiotensin II, vasopressin, and the overactivity of the sympathetic nervous system produce significant vasoconstriction in the extrasplanchnic organs, including the kidneys, but not in the splanchnic circulation, which is resistant to these vasoconstrictor stimuli owing to an increase in the local synthesis of vasodilators. The circulatory profile of a patient with decompensated cirrhosis, therefore, consists of a progressive decrease of effective arterial blood volume because of both an increase in splanchnic arterial vasodilatation and a decrease in cardiac output; a progressive compensatory activation of the rennin–angiotensin system, sympathetic nervous system, and antidiuretic hormone; and a progressive impairment of the perfusion of extrasplanchnic organs.

Because splanchnic arterial vasodilatation is the predominant mechanism by which splanchnic lymph formation is increased in cirrhosis, the pathogenesis of ascites can be satisfactorily explained on the basis of the changes in the arterial circulation induced by portal hypertension. This "forward" hypothesis considers that the accumulation of fluid within the peritoneal cavity is the consequence of the splanchnic arterial vasodilatation, which simultaneously produces a reduced effective arterial blood volume and a "forward" increase in splanchnic capillary pressure (Fig. 19.14). In patients with compensated cirrhosis or presinusoidal portal hypertension, the degree of portal hypertension and splanchnic arterial vasodilatation is moderate, the lymphatic system is able to return the excess of lymph produced in the hepatic and splanchnic area to the systemic circulation, and the arterial vascular underfilling is compensated by transient periods of sodium and water retention that increase the plasma volume and cardiac index and refill the dilated vascular bed. As cirrhosis progresses, however, portal hypertension and the secondary splanchnic arterial vasodilatation become progressively more intense and a critical point is

reached at which the consequences of splanchnic arterial vasodilatation can no longer be compensated by increasing lymph return, plasma volume, and cardiac output. The patients have effective hypovolemia and sodium and water retention, but this fluid is ineffective in compensating this impairment of circulatory function because it escapes from the intravascular compartment because of an imbalance between the formation and the reabsorption of lymph. The final consequence of both disorders is the continuous formation of ascites.





▪ **Figure 19.14** The "forward" theory of ascites formation.

During the initial stages of decompensated cirrhosis, sodium retention occurs despite normal levels of renin, aldosterone, and norepinephrine. It has been proposed that sodium retention at this period may be caused by mechanisms unrelated to a reduction in effective blood volume (reduced hepatic metabolism of some endogenous substance with sodium-retaining effect or a direct hepatorenal reflex) and may promote sodium retention. This is highly unlikely because these patients have hemodynamic characteristics identical to those of patients with ascites and high renin levels. The most likely explanation is that a still unknown mechanism extremely sensitive to changes in effective blood volume induces sodium retention at these early stages of decompensated cirrhosis. This mechanism would be more sensitive than that of the sympathetic nervous system and renin–angiotensin–aldosterone system and, consequently, would be stimulated earlier. Activation of the sympathetic nervous system and the renin–angiotensin–aldosterone system represents a further step and indicates more severe impairment of circulatory function as a consequence of the progression of the disease. Finally, the level of plasma antidiuretic hormone, the secretion of which is highly sensitive to small changes in serum osmolality but requires greater changes in effective blood volume, increases at later stages of the disease. This phenomenon explains why dilutional hyponatremia is a late event in the course of decompensated cirrhosis.

Management of Ascites in Cirrhosis

Bed rest, low sodium diet, and diuretics

The assumption of an upright posture associated with moderate physical exercise by patients with cirrhosis and ascites induces a marked stimulation of the renin–

angiotensin–aldosterone system and sympathetic nervous system (61). Therefore, from a theoretic point of view, bed rest may be useful in patients with poor response to diuretics. Because the natriuretic action of loop diuretics starts soon after administration and disappears approximately 3 hours later, bed rest should be adjusted to this time schedule. The effect of spironolactone lasts for more than 1 day and, therefore, is not important in planning bed rest.

Mobilization of ascites occurs when a negative sodium balance is achieved. In 10% to 20% of patients, those spontaneously excreting relatively high amounts of sodium in the urine, this can be obtained simply by reducing the sodium intake to 40 to 70 mEq/day (i.e., no salted food, no salt during cooking, no salt on the table). A greater reduction in sodium intake interferes with the nutrition of the patients and is not advisable (62). In most instances, a negative sodium balance cannot be achieved unless urinary sodium excretion is increased with diuretics. Even in these patients, sodium restriction is important because it reduces diuretic requirements. Patients responding satisfactorily to diuretics may be allowed to increase the sodium intake up to 70 to 100 mEq/day if they do not tolerate the standard low sodium diet. However, sodium restriction is essential in the care of patients responding poorly to diuretics. A frequent cause of "apparently" refractory ascites is inadequate sodium restriction. This should be suspected whenever ascites does not decrease despite a good natriuretic response to diuretics. Once ascites is mobilized, it is better to reduce the diuretic dosage than to increase sodium intake.

Furosemide and spironolactone are the diuretics most commonly used in the treatment of ascites in patients with cirrhosis. Furosemide, as do other loop diuretics (torsemide, ethacrynic acid, bumetanide), inhibits chloride and sodium reabsorption in the thick ascending limb of the loop of Henle but has no effect on the distal nephron (distal and collecting tubules). Furosemide is rapidly absorbed from the intestine, is highly bound to plasma proteins, and is actively secreted from the blood into the urine through the organic acid transport pathway in the proximal tubule. Once in the luminal compartment, furosemide is carried in the luminal fluid to the loop of Henle, where it inhibits the Na^+-$2Cl^-$-K^+ cotransport system located in the luminal membrane of the ascending limb cells, and sodium reabsorption occurs in this segment of the nephron. Because between 30% and 50% of the filtered sodium is reabsorbed in the loop of Henle, it is not surprising that furosemide has a high natriuretic potency. At high dosage, it can increase sodium excretion by up to 30% of the filtered sodium in healthy subjects. Furosemide also increases the synthesis of prostaglandin E_2 by the ascending limb cells. This effect is related to the natriuretic effect because nonsteroidal anti-inflammatory drugs reduce its natriuretic activity. The onset of action of furosemide is extremely rapid (within 30 minutes of oral administration), with the peak effect occurring within 1 to 2 hours and most natriuretic activity stopping 3 to 4 hours after administration.

Spironolactone undergoes extensive metabolism that produces numerous biologically active compounds, the

most important one being canrenone. These aldosterone metabolites are tightly bound to plasma proteins from which they are released slowly to the kidney and other organs. Spironolactone metabolites act by competitively inhibiting the tubular effect of aldosterone on the distal nephron. This hormone enters the collecting tubule through the basolateral membrane and interacts with a cytosolic receptor. The aldosterone receptor complex is translocated to the nuclei and

interacts with specific DNA sequences, stimulating the release of messenger ribonucleic acid and the synthesis of sodium channels, which are inserted into the luminal membrane, and the transporter Na^+/K^+-adenosine triphosphatase (ATPase), which activates the extrusion of sodium from the intracellular space into the peritubular interstitial space. The effect of this transporter together with the activation of potassium channels in the luminal membrane is the predominant mechanism of the kaliuretic effect of aldosterone. Spironolactone metabolites also enter the basolateral membrane in the collecting tubule and interact with the cytosolic receptor, but the complex spironolactone metabolite receptor is unable to interact with DNA. Therefore, spironolactone acts as a specific antagonist of aldosterone. The half-life of the aldosterone-induced proteins and of spironolactone metabolites is relatively prolonged, explaining the lag of 2 to 3 days between the initiation or the discontinuation of spironolactone treatment and the onset or the end of the natriuretic effect, respectively. Spironolactone metabolism is impaired in cirrhosis, such that the terminal half-life of spironolactone metabolites is increased in this condition. Because the amount of sodium reabsorbed in the collecting tubule is low, spironolactone and other distal diuretics (e.g., triamterene, amiloride) have a much lower natriuretic potency than furosemide. They are able to increase sodium excretion by up to 2% of the filtered sodium.

The administration of furosemide at relatively high doses (80 to 160 mg/day) to nonazotemic patients with cirrhosis and ascites gives rise to a satisfactory natriuretic response in only 50% of cases. In contrast, most of these patients respond to spironolactone at doses of 150 to 300 mg/day (55) (Table 19.4). The mechanism of this resistance to the natriuretic effect of furosemide is mainly pharmacodynamic. Most of the sodium not reabsorbed in the loop of Henle by the action of furosemide is subsequently reabsorbed in the distal nephron by the action of aldosterone. Patients responding to furosemide are those with normal or only moderately increased plasma aldosterone levels. Patients with marked hyperaldosteronism usually do not respond to this drug. The response to spironolactone depends on the degree of hyperaldosteronism. Patients with a normal or slightly increased plasma concentration of aldosterone usually respond to low doses of spironolactone (100 to 150 mg/day), but as much as 300 to 400 mg/day may be needed to antagonize the tubular effect of aldosterone in patients with marked hyperaldosteronism. The basic drug for the treatment of ascites, therefore, is spironolactone. Simultaneous administration of furosemide and spironolactone increases the natriuretic effect of both agents and reduces the incidence of hypo- or hyperkalemia that can occur when these drugs are given alone.

Table 19.4. Comparison of the Efficacy of Furosemide and Spironolactone in Nonazotemic Cirrhosis with Ascites

	Positive response	Negative response	Total
Furosemide	11	10[a]	21
Spironolactone	18	1[b]	19

χ^2=6.97; P < 0.01.
[a]Nine cases responded later to spironolactone.
[b]This case did not respond later to furosemide.
From (63) Pérez-Ayuso RM, Arroyo V, Planas R, et al. Randomized comparative study of efficacy of furosemide versus spironolactone in nonazotemic cirrhosis with ascites. *Gastroenterology* 1984;84:961–968, with permission.

Two different diuretic approaches can be used in patients with cirrhosis and ascites. The step-care approach (64) consists of the progressive implementation of the therapeutic measures currently available, starting with sodium restriction. If ascitic volume does not decrease (as measured by loss of body weight), spironolactone is given at increasing doses (starting with 100 mg/day; if no response is seen within 4 days, increasing to 200 mg/day; and if no response is seen, further increasing to 400 mg/day). When there is no response to the highest dose of spironolactone, furosemide is added, also by increasing the dosage every 2 days (40 to 160 mg/day). The second approach is the combined treatment. It begins with the simultaneous administration of sodium restriction, spironolactone 100 mg/day, and furosemide 40 mg/day. If the diuretic response is insufficient after 4 days, the dose is increased to 200 mg/day and 80 mg/day, respectively. For patients who do not respond despite the increase in dosage, spironolactone and furosemide are increased to 400 mg/day and 160 mg/day, respectively. A recent randomized controlled trial has shown that the step-care and the combined treatment approaches are similar in terms of response rate, rapidity of ascites mobilization, and incidence of complications (65). There is a general agreement that patients not responding to 160 mg/day of furosemide and 400 mg/day of spironolactone will not respond to higher doses of these diuretics. For patients receiving the combined treatment with an exaggerated response,

diuretic administration should be adjusted with a reduction in the dose of furosemide. The goal of diuretic treatment should be to achieve a weight loss of 300 to 500 g/day in patients without peripheral edema and 500 to 1,000 g/day in patients with peripheral edema. Once ascites is mobilized, diuretic treatment should be reduced to keep the patients free of ascites. The most important predictor of diuretic response in patients with cirrhosis and ascites is the degree of impairment of circulatory and renal function. Patients with increased serum creatinine levels (>1.2 mg/dL; upper normal limit), dilutional hyponatremia (serum sodium concentration <130 mEq/L), or intense hyperaldosteronism need a high diuretic dosage or do not respond to the highest doses of furosemide and spironolactone.

The major complications associated with diuretic management of cirrhosis with ascites are renal failure, hyponatremia, and hepatic encephalopathy (55). Approximately 20% of patients with cirrhosis and ascites have marked renal impairment (increased blood urea nitrogen and serum creatinine levels), which is usually moderate and always reversible after diuretic withdrawal. It is caused by

a reduction in intravascular volume caused by an imbalance between the fluid loss induced by the diuretic treatment and the reabsorption of ascitic fluid into the general circulation, which varies greatly from patient to patient. The incidence of diuretic-induced renal failure is lower among patients with ascites and peripheral edema than among those without edema because there is no limitation in the reabsorption of peripheral edema into the general circulation; therefore, it compensates any insufficient reabsorption of ascites.

Hyponatremia secondary to impairment of the renal ability to excrete free water also occurs in 20% of patients with cirrhosis and ascites managed with diuretics. Two mechanisms are involved in this complication. The first is related to the reduction in intravascular volume, which stimulates baroreceptors and the secretion of antidiuretic hormone. The second is related to the action of furosemide. Free water (water free of solutes) is generated within the kidney by the active reabsorption of chloride and sodium without the concomitant reabsorption of water from the water-impermeable ascending limb of the loop of Henle. The hypotonic urine generated by this process is maintained throughout the distal nephron if antidiuretic hormone secretion is inhibited, for example, after a water load. Furosemide interferes with the generation of free water because it inhibits chloride and sodium reabsorption in the ascending limb of the loop of Henle. In patients with advanced cirrhosis, who have a spontaneous severe reduction in free water excretion caused by homeostatic nonosmotic hypersecretion of antidiuretic hormone, any additional impairment of renal water metabolism, because of either further stimulation of antidiuretic hormone secretion or interference with the diluting process of the urine in the loop of Henle, can precipitate the development of severe hyponatremia.

The most severe complication of diuretic therapy for cirrhosis with ascites is hepatic encephalopathy, which has been reported to occur in 25% of cases (55). The mechanism is unknown. It has been suggested that it may be caused by an increase in renal production of ammonia. Accentuation of the cerebral vasoconstriction already present in these patients secondary to the reduction in intravascular volume may be a contributory event.

Other complications of use of diuretics to manage cirrhosis include hyperkalemia and metabolic acidosis in patients with renal failure treated with high doses of spironolactone, hypokalemia in patients treated with high doses of furosemide and no or low doses of spironolactone, gynecomastia in patients receiving spironolactone, and muscle cramps. Muscle cramps are clearly related to the reduction in intravascular volume because they occur in patients with severe baseline circulatory dysfunction and can be prevented by means of plasma volume expansion with albumin. Oral administration of quinine also reduces the frequency of diuretic-induced muscle cramps (57).

Therapeutic paracentesis

Treatment with low sodium diet and diuretics is effective in mobilizing ascites in cirrhosis. However, it has several limitations. First, approximately 10% to 20% of patients do not respond to diuretics (diuretic-resistant ascites). Second, diuretic treatment is frequently associated with complications, particularly when high doses of diuretics have to be used. Finally, the mobilization of ascites with diuretics is a slow process. This problem is not relevant to the care of patients with moderate ascites, who are usually treated as outpatients.

In 1987 the demonstration that large-volume paracentesis associated with

plasma volume expansion is a rapid, effective, and safe treatment of ascites in cirrhosis has considerably simplified the treatment of patients admitted to the hospital with tense ascites (66). Therapeutic paracentesis is considered the best therapy for tense ascites in cirrhosis (62). It considerably shortens hospital stay and, therefore, the cost of treatment, and the incidence of complications during hospitalization is significantly lower among patients undergoing paracentesis than among those treated with diuretics (66,67,68).

Although paracentesis is a simple procedure, several precautions should be taken to avoid complications. Therapeutic paracentesis can be performed either as repeated large-volume paracentesis (4 to 6 L/day until complete disappearance of ascites) or as total

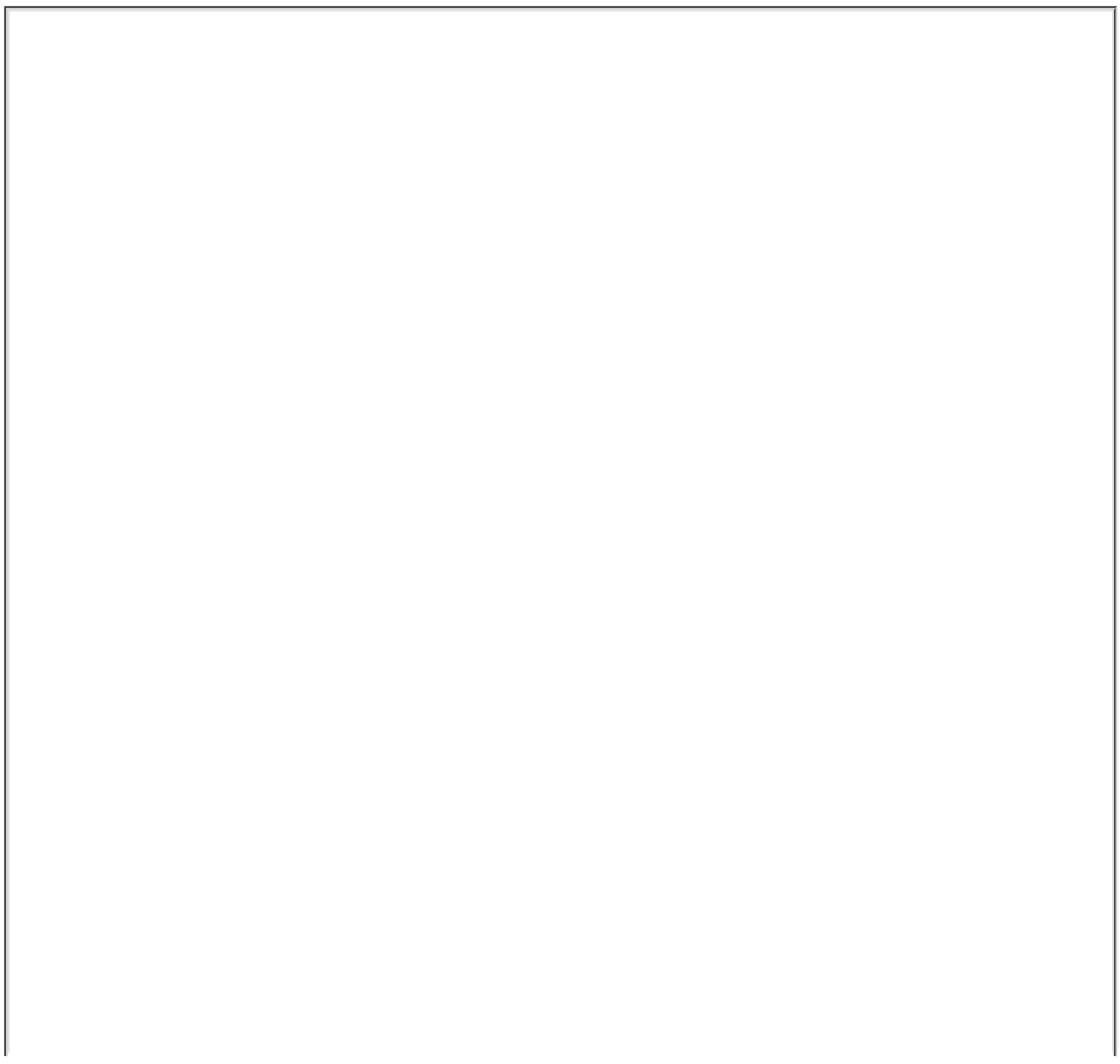
paracentesis (complete removal of ascites in only one paracentesis session). Total paracentesis is the best method because it is faster and associated with lower incidence of local complications (49,57,58). Ascites leakage through the skin or within the abdominal wall is relatively frequent after partial paracentesis because a significant volume of ascites remains in the peritoneal cavity after the procedure. On the other hand, although complications related to the insertion of the needle are exceptional, the incidence increases with the number of taps. Paracentesis should be performed under strictly sterile conditions with specially designed needles. We use a modified Küss needle, which is a sharp, pointed, blind, metal needle within a 7-cm long, 17-gauge, metal, blunt-edged cannula with side holes. With the patient under local anesthesia, the needle is inserted into the left lower abdominal quadrant. The inner part is removed, and the cannula is connected to a large-capacity suction pump. The physician should remain at the bedside throughout the procedure. With this technique, the duration of treatment ranges from 30 to 60 minutes, depending on the amount of ascitic fluid removed. Total paracentesis procedures are finished when the flow from the cannula becomes intermittent despite gentle mobilization of the cannula within the peritoneal cavity and turning the patient to the left side. Peripheral edema is rapidly reabsorbed after the mobilization of ascites in most patients and usually disappears within the first 2 days of treatment. Most of the fluid goes to the abdominal cavity as ascites. It is therefore not infrequent for patients with marked peripheral edema to need a second procedure after complete mobilization of ascites at the initial paracentesis. Patients treated by means of repeated large-volume paracentesis should recline for 2 hours on the side opposite the paracentesis site to prevent the leakage of ascitic fluid. The modified Küss needle and specific kits for paracentesis that include the needle are now available commercially.

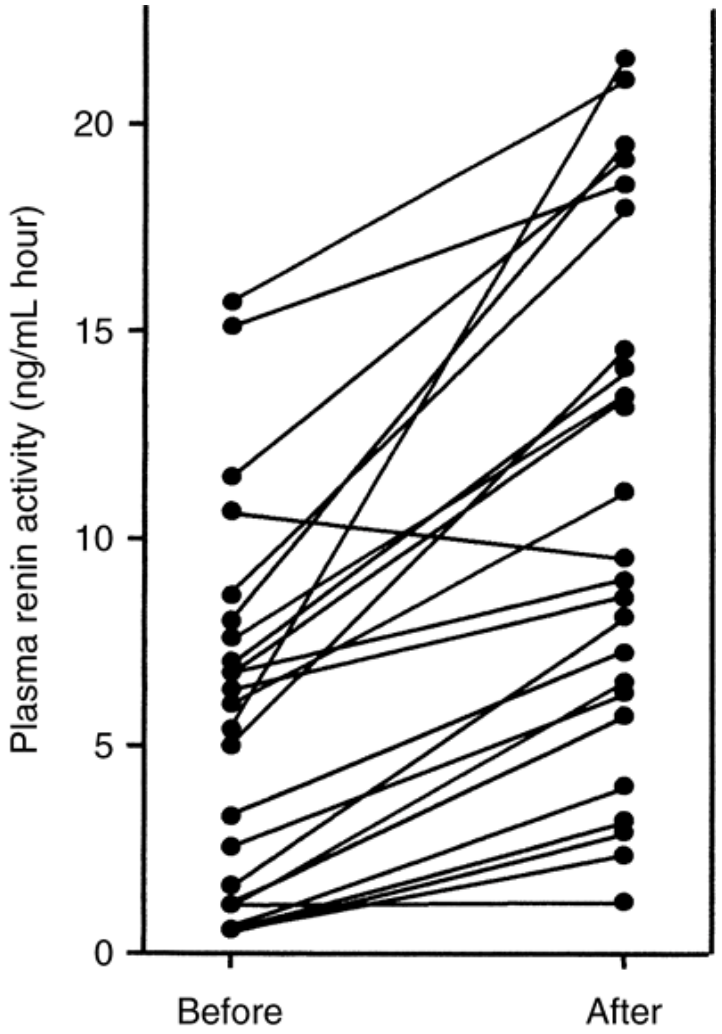
When paracentesis is performed without plasma volume expansion, there are no apparent major changes in circulatory function. Arterial pressure decreases slightly, but this also occurs when paracentesis is performed with plasma volume expansion. The pulse rate does not increase, and the patient does not experience any symptoms other than those related to the disappearance of ascites (67,69). In addition, if serum creatinine and serum electrolytes are measured within the first days of performance of paracentesis, no changes are observed in most patients. For this reason, some investigators consider that therapeutic paracentesis does not adversely affect circulatory function and that, consequently, plasma volume expansion is not necessary in the care of patients with cirrhosis and ascites treated with this procedure.

Many studies indicate that marked changes in circulatory function occur after

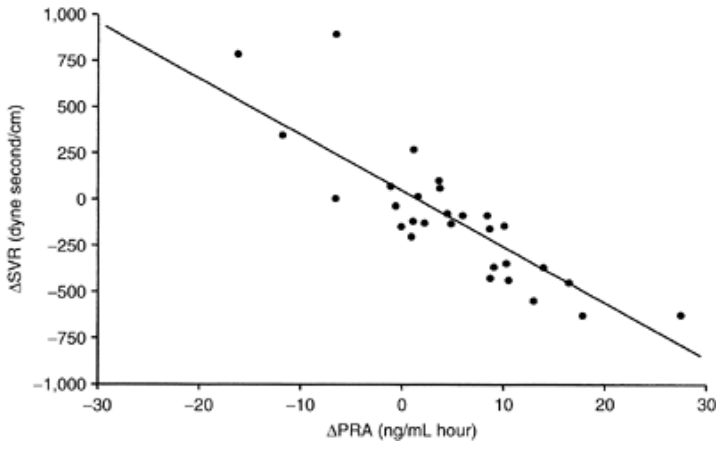
therapeutic paracentesis (59). Immediately after paracentesis, circulatory function improves, with a marked increase in cardiac output and stroke volume, a reduction in cardiopulmonary pressure, and a suppression of the renin–angiotensin and sympathetic nervous systems (59). These effects, which persist for approximately 12 hours and have been attributed to mechanical factors (i.e., reduction in intrathoracic pressure and increase in venous return), are followed by opposing hemodynamic changes, including a reduction in cardiac output to baseline value and marked activation of the renin–angiotensin (Fig. 19.15) and sympathetic nervous systems over the corresponding levels before paracentesis (69). Renal function also improves during the first hours after paracentesis and may worsen 24 to 48 hours after the procedure. The impairment of circulatory function induced by paracentesis is not related, as proposed initially, to a decrease in circulating blood volume secondary to a rapid reaccumulation of ascites, but rather to an accentuation of the arterial vasodilatation already present in these patients (Fig. 19.16). The mechanism by which paracentesis induces reduction of peripheral vascular resistance and the site where this vasodilatation occurs are unknown, although it is probably in the splanchnic circulation. An important observation is that the circulatory dysfunction induced by paracentesis is not

spontaneously reversed (68). Once plasma renin activity and plasma norepinephrine concentration increase, they remain elevated throughout the course of the disease. The cause of this phenomenon is unknown.





• **Figure 19.15** Plasma renin activity before and after therapeutic paracentesis without albumin infusion. (From Ginès P, Tito L, Arroyo V, et al. Randomized comparative study of therapeutic paracentesis with and without intravenous albumin in cirrhosis. *Gastroenterology* 1988;94:1493–1502, with permission.)

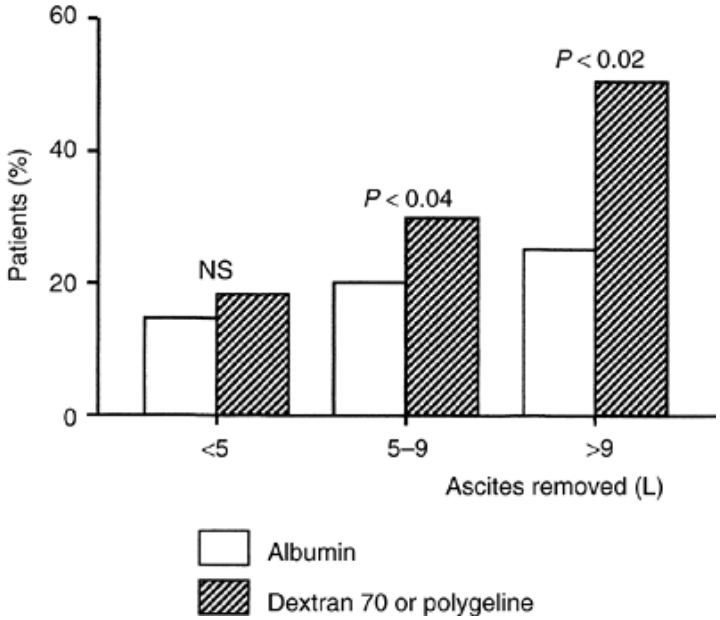


▪ **Figure 19.16** Direct negative correlation between the increase in plasma renin activity (ΔPRA) and the decrease in systemic vascular resistance (ΔSVR) after therapeutic paracentesis in cirrhosis. (From Ruiz del Arbol L, Monescillo A, Jimenez W, et al. Paracentesis-induced circulatory dysfunction: mechanism and effect on hepatic hemodynamics in cirrhosis. *Gastroenterology* 1997;113:579–586, with permission.)

Plasma renin activity is a sensitive marker of circulatory function and is the parameter used to detect impairment of circulatory function after paracentesis in most studies. Paracentesis-induced circulatory dysfunction has been defined as a 50% increase in plasma renin activity over baseline on the sixth day after treatment up to a value greater than 4 ng/mL hour (upper normal limit) (50, 59, 60). According to this criterion, the incidence of spontaneous circulatory dysfunction among patients with cirrhosis admitted to hospital because of tense ascites and not receiving any treatment during 1 week of hospitalization was 16% (unpublished observations obtained in 56 patients). The incidence of paracentesis-induced circulatory dysfunction has been estimated to be 75% among patients not undergoing plasma volume expansion, 33% to 38% in patients receiving polygeline (saline solution, 8 g/L of ascitic fluid removed), dextran 70 (dextrose solution, 8 g/L of ascitic fluid removed, or saline), and 11% to 18% among patients receiving albumin (salt-poor solution, 8 g/L of ascitic fluid removed) (68). Similar findings have been reported in a recent trial comparing albumin versus saline in patients with ascites treated by total paracentesis (70). The incidence of paracentesis-induced circulatory dysfunction was 33.3% in patients receiving saline and 11.4% in those receiving albumin.

In the care of patients undergoing plasma volume expansion and treated by total paracentesis, the amount of ascitic fluid volume removed is a predictor of paracentesis-induced circulatory dysfunction (Fig. 19.17). When the amount of ascitic fluid removed is less than 5 L, the

incidence of circulatory dysfunction is similar among patients treated with albumin and those treated with synthetic plasma expanders (16% vs. 18%). However, when the amount is between 5 and 9 L, the incidence of circulatory dysfunction is higher among patients receiving synthetic plasma expanders (19% vs. 30%). Differences are particularly marked when the volume of the paracentesis is greater than 9 L. In the latter case, the incidence of paracentesis-induced circulatory dysfunction is 52% among patients receiving synthetic plasma expanders (68).



▪ **Figure 19.17** Relationship between the rate of circulatory dysfunction following paracentesis, the volume of ascites removed, and the type of plasma expander used. NS, not significant. (From Ginès A, Fernandez-Esparrach G, Monescillo A, et al. Randomized trial comparing albumin, dextran 70, and polygeline in cirrhotic patients with ascites treated by paracentesis, *Gastroenterology* 1996;111:1002–1010, with permission.)

These data indicate the following: (a) Paracentesis-induced circulatory dysfunction is frequent when the plasma volume is not expanded, (b) plasma volume expansion with synthetic colloids is effective in reducing the incidence of circulatory dysfunction after paracentesis, (c) plasma volume expansion with albumin almost totally prevents paracentesis-induced circulatory dysfunction, (d) among patients with an ascitic fluid volume of less than 5 L, the incidence of paracentesis-induced circulatory dysfunction is low and independent of the type of the plasma expander used, (e) when the amount of ascitic fluid volume removed is over 5 L, the incidence of circulatory dysfunction increases with the volume of paracentesis in patients receiving synthetic plasma expanders but not in those receiving albumin.

Despite being asymptomatic, paracentesis-induced circulatory dysfunction adversely affects the clinical course of the disease. The incidence of hyponatremia (3.8% vs. 17%) and renal impairment (0% vs. 11%) within few days of paracentesis is significantly lower among patients receiving albumin infusions than among those not receiving plasma expanders. The time to first readmission to hospital is significantly shorter for patients with circulatory dysfunction after paracentesis than among those who do not have this complication. Finally, the probability of survival is also lower among patients with circulatory dysfunction after paracentesis (68).

The mechanism by which deterioration in circulatory function impairs the clinical course and the prognosis for patients with cirrhosis and ascites is probably multifactorial. Circulatory dysfunction is associated with an increase in the

circulating levels of vasoconstrictors, which impairs renal hemodynamics and the renal response to diuretics. Angiotensin II and norepinephrine are important mediators of HRS, which are associated with a poor survival. These substances also induce vasoconstriction of intrahepatic vascular resistance, which may reduce liver perfusion, impair hepatic function, and increase portal pressure. These changes may further deteriorate circulatory function and create vicious circles that accelerate the course of the disease. One study has shown that the hepatic venous pressure gradient (an estimation of the intrahepatic vascular resistance) increases after paracentesis in patients with circulatory dysfunction but not in patients who do not have this complication (69).

There is substantial evidence indicating that paracentesis-induced circulatory dysfunction is a relevant complication that should be prevented. The best way to do this is to expand the plasma volume with albumin when the volume of ascitic fluid removed is more than 5 L. When the volume is less than 5 L, less expensive synthetic plasma expanders can be used. The amount of albumin given in most centers is 8 g/L of ascitic fluid removed, which represents the approximate amount of albumin removed with the paracentesis. Fifty percent of the dose is infused immediately after paracentesis and 50% after 6 hours. The patient may then leave the hospital with diuretics to prevent the reaccumulation of ascites. Patients with normal blood urea nitrogen and serum creatinine levels require a standard diuretic dosage (200 mg/day of spironolactone or 40 mg/day of furosemide plus 100 mg/day of spironolactone). Higher diuretic dosages, however, are required in patients with abnormal blood urea nitrogen or serum creatinine concentration, or in patients with ascites that is refractory before treatment.

Management of refractory ascites

According to the International Ascites Club, the term *refractory ascites* applies to the ascites that cannot be mobilized or the early recurrence of which (i.e., after therapeutic paracentesis) cannot be prevented by medical therapy (70). There are two subtypes of refractory ascites. Diuretic-resistant ascites is the type that cannot be mobilized (loss of body weight less than <200 g/day after 4 days) or the early recurrence of which cannot be prevented because of a lack of response to dietary sodium restriction (approximately 50 mEq/day) and intensive diuretic treatment (spironolactone 400 mg/day plus furosemide 160 mg/day). Diuretic-intractable ascites is the type that cannot be mobilized or the early recurrence of which cannot be prevented because of the development of diuretic-induced complications that precludes the use of an effective diuretic dosage (e.g., hepatic encephalopathy in the absence of any other precipitating cause, increase in serum creatinine by >100% to a value >2 mg/dL, decrease in serum sodium level by >10 mEq/L to a concentration <125 mEq/L, and decrease of serum potassium level to <3 mEq/L or an increase to >6 mEq/L despite appropriate measures to normalize potassium concentration). Recidivant ascites is the type that recurs frequently (on three or more occasions within a 12-month period) despite dietary sodium restriction and adequate diuretic dosage. This condition should not be considered as refractory ascites.

Most patients with cirrhosis who have diuretic-resistant ascites have type 2 HRS (serum creatinine level >1.5 mg/dL) or lesser despite marked degrees of impairment of renal perfusion and GFR (serum creatinine level between 1.2 and 1.5 mg/dL). It has

been estimated that a serum creatinine level greater than 1.2 mg/dL in patients with cirrhosis and ascites reflects a decrease of renal blood flow and GFR greater than 50% with respect to values in healthy persons. The most important mechanisms of refractory ascites are (a) impairment of the access of diuretics to the effective sites on the tubular cells due to the renal hypoperfusion and (b) reduced delivery of sodium to the ascending limb of the loop of Henle and the distal nephron secondary to the low GFR and an excessive sodium reabsorption in the proximal tubule. Inadequate sodium restriction or the use of nonsteroidal anti-inflammatory drugs should be ruled out in the evaluation of any patient with the presumptive diagnosis of diuretic-resistant ascites.

Three different treatments can be used for the management of patients with cirrhosis and refractory ascites: Peritoneovenous shunting, TIPS, and therapeutic paracentesis (71,72). Peritoneovenous shunting was the first treatment specifically designed for patients with refractory ascites. LeVeen et al. introduced the first prosthesis in 1974. It consists of a perforated intra-abdominal tube connected through a one-way pressure-sensitive valve to a second tube that traverses the subcutaneous tissue up to the neck, where it enters the internal jugular vein. The tip of the intravenous tube is located in the superior vena cava near the right atrium. Insertion of a LeVeen shunt is technically simple and can be performed under local anesthesia. It is advisable to remove most of the ascitic fluid before the insertion of the prosthesis to avoid early complications related to the massive passage of ascites to the general circulation (e.g., pulmonary edema, variceal hemorrhage, and severe intravascular coagulation). Prophylactic administration of antistaphylococcal antibiotics before and after surgery is also recommended. Although the LeVeen shunt is the most widely used, other types, such as the Denver shunt, are available. However, they do not improve the results obtained with the initial prosthesis.

The shunt produces a sustained expansion of the circulating blood volume by the continuous passage of ascitic fluid from the abdominal cavity to the systemic circulation; a marked suppression of the plasma levels of renin, norepinephrine, and antidiuretic hormone; and an increased response to diuretics. Therefore, it is a rational therapy for refractory ascites (10). Unfortunately, obstruction of the shunt is common and occurs in approximately 40% of patients within the first postoperative year; it is usually due to the deposition of fibrin either in the valve or around the intravenous catheter, thrombotic obstruction of the venous limb of the prosthesis, or thrombosis of the superior vena cava. Although thrombosis of the vena cava is usually incomplete, total occlusion can occur, resulting in the development of a superior vena cava syndrome. Shunt occlusion requires reoperation, removal of the obstructed shunt, and insertion of a new prosthesis. Another long-term complication of peritoneovenous shunting is small-bowel obstruction, which occurs in approximately 10% of patients. Small intestinal obstruction is caused by marked intraperitoneal fibrosis and can make further intra-abdominal procedures, such as liver transplantation, impossible.

The reintroduction of therapeutic paracentesis has markedly reduced the use of peritoneovenous shunting in patients with refractory ascites. Results of two randomized controlled trials have been published in which paracentesis was compared with use of a LeVeen shunt in the care of these patients. Although shunting was clearly superior in the long-term control of ascites, it had no effect on the course of the disease. Patients from both therapeutic groups did not differ in the time to first readmission to the hospital during the follow-up and survival (Table 19.5). Furthermore, frequent reoperations were needed because of shunt

obstruction (10). These data led the International Ascites Club to propose that paracentesis is preferred to peritoneovenous shunting for the management of refractory ascites.

TIPS is the most recent treatment introduced for the management of refractory ascites. It works as a side-to-side portacaval shunt and, from a theoretic point of view, it should correct the two principal mechanisms in the pathogenesis of ascites (71). By doing so, it should suppress the endogenous vasoconstrictor system, improve renal perfusion and GFR, and increase the response to diuretics. On the other hand, by decompressing both the splanchnic and the hepatic microcirculation, TIPS should decrease the formation of lymph both in the liver and in the other splanchnic organs.

A review of the records of the first 358 reported patients with refractory ascites treated with TIPS clearly indicated that this therapeutic procedure is extremely effective in improving circulatory and renal function and in managing ascites in these patients (71). TIPS induces a marked increase in cardiac output, a decrease in systemic vascular resistance, and an elevation in right atrial pressure, pulmonary artery pressure, and pulmonary wedge pressure (59,60). These changes, which

are similar to those after peritoneovenous shunting, are probably caused by an increase in venous return resulting from the presence of portacaval fistula. The decrease in systemic vascular resistance, which is also a constant feature in patients treated by peritoneovenous shunting, is probably a physiologic response to accommodate the increase in cardiac output.

Table 19.5. Peritoneovenous Leveen Shunt Versus Therapeutic Paracentesis in the Management of Refractory Ascites: Efficacy, Associated Complications, and Survival

	Paracentesis (*n* = 38)	LVS (*n* = 42)
Ascites episodes	125	38
LVS obstruction[a]	—	40%
Time in hospital (days)	48 ± 8	44 ± 6
Survival[a]	57%	44%

[a]1-year probability.
LVS, LeVeen shunt.

Because it increases the hyperdynamic circulation, it has been suggested that TIPS impairs the systemic hemodynamics in cirrhosis. However, results of studies of the effects of TIPS on the endogenous vasoactive systems do not support this concept. The results indicate that effective arterial blood volume is markedly improved after TIPS insertion in patients with cirrhosis and ascites. As indicated

earlier, the maintenance of arterial pressure in patients with advanced cirrhosis and ascites is critically dependent on a marked overactivity of the renin–angiotensin system, sympathetic nervous system, and antidiuretic hormone. If TIPS enhances arterial vasodilatation, a further increase in the degree of stimulation of these vasoconstrictor systems should occur. In contrast, TIPS insertion is associated with marked suppression of the plasma levels of renin, aldosterone, norepinephrine, and antidiuretic hormone (59,60). Suppression of the renin–angiotensin–aldosterone system occurs within the first week of TIPS insertion and persists during the follow-up period. Suppression of norepinephrine and antidiuretic hormone seems to require a longer period of time.

Deterioration in circulatory function should also be associated with a further impairment of renal function after TIPS insertion; however, this process induces a rapid increase in urinary sodium excretion, which is already observed within the first 1 to 2 weeks and persists during the follow-up period (59,60). A significant increase in serum sodium concentration and GFR is also observed, indicating an improvement in renal perfusion and free water clearance. However, these latter changes require 1 to 3 months to occur.

TIPS induces a marked decrease in the portacaval gradient. In the aforementioned review of the care of 358 patients with refractory ascites treated by TIPS, the mean decrease was from 20.9 to 10 mm Hg (60). Portal venous pressure also decreased markedly, from 29.4 to 21.8 mm Hg. However, TIPS only partially decompresses the portal venous system; portal venous pressure in most healthy subjects is less than 5 mm Hg. Although suppression of the renin–aldosterone system is evident, the plasma levels of renin and aldosterone do not decrease to normal levels. Improvement in splanchnic and systemic hemodynamics is associated with the disappearance of ascites or partial response (no need for paracentesis) in most patients. Only 10% of cases do not respond to TIPS. Ascites characteristically resolves slowly (within 1 to 3 months). Continuous diuretic treatment is required in more than 95% of cases, either for the management of ascites or to reduce the peripheral edema that frequently occurs in patients treated with TIPS. The persistence of portal hypertension and hyperaldosteronism may be the explanation for this phenomenon.

Hepatic encephalopathy is the most important complication among patients with cirrhosis and refractory ascites managed with TIPS (60). More than 40% of patients have this complication. In most cases hepatic encephalopathy responds to standard therapy. However, it occasionally requires a decrease in stent size. Although hepatic encephalopathy before insertion of TIPS is a predictor of encephalopathy after its insertion, new or worsening hepatic encephalopathy develops in approximately 30% of cases. Shunt dysfunction is also a major problem, occurring in approximately 40% of cases within the first year. This is an important limitation of TIPS that necessitates frequent retreatments. The 1-year probability of survival among patients with cirrhosis and refractory ascites treated with TIPS is extremely poor. Early mortality (within 30 days of insertion of TIPS) is approximately 12% and late mortality is 40%. Predictors of survival are the Child-Pugh score, age, and the presence of HRS before TIPS insertion (60).

Five randomized controlled trials have been reported comparing TIPS and therapeutic paracentesis (73,74,75,76,77). Two included patients with recidivant and refractory ascites, and three included patients with only refractory ascites. The five trials clearly showed that TIPS was better than paracentesis in the long-term control of ascites. Three trials showed significantly higher incidence of

hepatic encephalopathy in patients treated with TIPS. An improvement in survival in the TIPS group was observed only in the trials including patients with recidivant ascites. The total time in hospital during follow-up was similar in both groups owing to the high incidence of shunt obstruction requiring new hospitalization for treatment of complications related to portal hypertension and/or restenting (Table 19.6). In one of these trials the quality of life was assessed and changes were similar in the two therapeutic groups (78). These results indicate that TIPS changes the course of cirrhosis from ascites to hepatic encephalopathy without improving the overall results of paracentesis in relation to length of hospitalization and survival.

Treatment of Patients with Cirrhosis, Ascites, and Hyponatremia or Hepatorenal Syndrome

Hyponatremia in patients with cirrhosis and ascites is usually asymptomatic, even in those with markedly reduced serum sodium concentration. On the other hand, it does not contraindicate diuretic treatment

because most patients respond to treatment without a further reduction in serum sodium concentration. Therefore, the use of aggressive procedures (e.g., peritoneovenous shunting, TIPS) for the treatment of hyponatremia is not justified. The intravenous administration of sodium chloride may produce a transient increase in serum sodium concentration but at the expense of increasing the rate of ascites formation. Finally, water restriction is difficult to carry out and is rarely effective. Therefore, at present there is no treatment for dilutional hyponatremia in cirrhosis. However, the future is very promising. Several specific antagonists of the renal effect of antidiuretic hormone (V2 antagonists) have been developed by different pharmaceutical companies and tested for treatment of patients with cirrhosis, ascites, and dilutional hyponatremia (11,12,79,80). These agents produce a marked increase in urine volume without a concomitant increase in urine sodium and solute excretion; this effect is associated with a significant increase in serum sodium concentration and serum osmolality in most patients. There are, however, patients in whom hyponatremia is refractory to aquaretic drugs (11), indicating that mechanisms other than antidiuretic drugs play an important role in the pathogenesis of free water retention in cirrhosis. The aquaretic drugs, therefore, will be important for the management of patients with cirrhosis. Potential indications would be not only the treatment of spontaneous dilutional hyponatremia but also the prevention and treatment of diuretic-induced hyponatremia.

Table 19.6. Transjugular Intrahepatic Portacaval Shunt Versus Paracentesis for Refractory Ascites—Summary of Studies

	Type of ascites	Control of ascites	Hepatic encephalopathy	Survival
Lebrec et al. (73)	Refractory	Better with TIPS	No difference	Worse with TIPS

Rössle et al. (74)	Refractory and recidivant	Better with TIPS	No difference	Better with TIPS
Ginès et al. (76)	Refractory	Better with TIPS	Worse with TIPS	No difference
Sanyal et al. (77)	Refractory	Better with TIPS	Worse with TIPS	No difference
Salerno et al. (75)	Refractory and recidivant	Better with TIPS	Worse with TIPS	Better with TIPS

TIPS, transjugular intrahepatic portacaval shunt.

Table 19.7. Effects of 1- to 2-Week Treatment with Ornipressin or Terlipressin Plus Albumin on Mean Arterial Pressure, Plasma Renin Activity, Norepinephrine, and Serum Creatinine Levels in Type 1 Hepatorenal Syndrome

	Baseline (*n* = 15)	**Day 7 (*n* = 9)**	**Day 14 (*n* = 7)**
MAP (mm Hg)	70 ± 8	77 ± 9	79 ± 12
PRA (ng/mL h)	15 ± 15	2 ± 3	1 ± 1
NE (pg/mL)	1,257 ± 938	550 ± 410	316 ± 161
Creatinine (mg/dL)	3 ± 1	2 ± 1	1 ± 1

Normal values: Plasma renin activity <1.4 ng/mL h; NE <250 pg/mL.
P < 0.001 for all values (analysis of variance [ANOVA]).
MAP, mean arterial pressure; PRA, plasma renin activity; NE, norepinephrine concentration.
From Guevara M, Gines P, Fernandez-Esparrach G, et al. Reversibility of hepatorenal syndrome by prolonged administration of ornipressin and plasma volume expansion. *Hepatology* 1998;27:35–41, and from Uriz J,

Ginès P, Cordenas A, et al. Terlipressin plus albumin infusion: an effective and safe therapy of hepatorenal syndrome. *J Hepatol* 2000;33:43–48, with permission.

For many years there has been no effective therapy for HRS, a severe complication. Expansion of plasma volume, administration of renal vasodilatory drugs (e.g., dopamine, prostaglandins), and insertion of a peritoneovenous shunt fail to produce a sustained increase in renal perfusion and GFR in these patients. However, the situation has changed completely during the last few years. Results of several studies show that long-term (1 to 2 weeks) simultaneous administration of albumin and vasoconstrictors (e.g., ornipressin, terlipressin, octreotide plus midodrine, or norepinephrine) to patients with severe type 1 HRS induces a normalization of plasma renin activity and a marked suppression of the plasma level of norepinephrine. These findings

indicate improvement in circulatory function (Table 19.7) (6,7) associated with normalization in serum creatinine and serum sodium concentration and a marked increase in GFR. The results of these studies also strongly suggest that reversal of HRS is associated with an increased survival (Table 19.8). TIPS is also effective in the treatment of patients with type 1 HRS (81,82). Improvement in renal function with TIPS occurs in approximately 75% of cases. Most important, survival rate also improves.

Table 19.8. Treatment of Hepatorenal Syndrome with Vasoconstrictors and Albumin and with Standard Medical Therapy: Review of 19 Studies (6,7,83,84,85,86,87,88,89,90,91,92,93,94,95,96)

	Group 1 (*n* = 155)	Group 2 (*n* = 137)	MCFS (*n* = 99)
Reversal of HRS	61.7%	2.9%	58%
HRS recurrence	20%	—	—
Survival 1 mo	41.6%	3%	40%
Survival 3 mo	30%	0%	22%

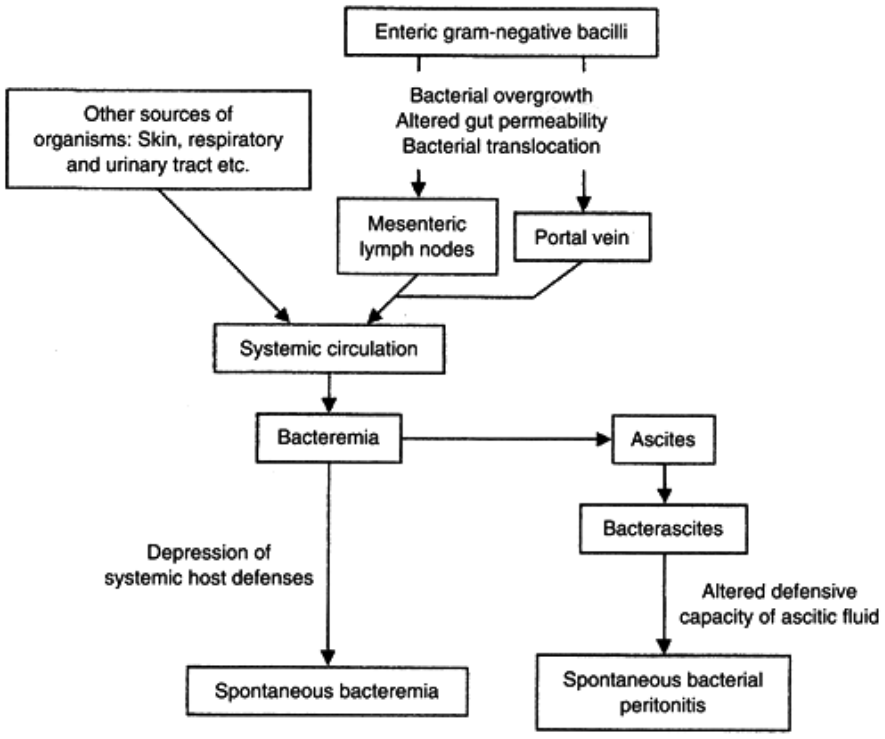
Liver transplantation	12.3%	—	13%

Group 1: It includes 12 pilot studies assessing treatment with vasoconstrictors (e.g., ornipressin, terlipressin, amidodrine, or noradrenaline) associated with the infusion of albumin in patients with hepatorenal syndrome (most with type 1 HRS).
Group 2: It includes six studies in patients with HRS (most with type 1 HRS) treated with standard medical therapy (volume expansion alone or associated with dopamine).
MCFS: Multicenter French Study reviewing 99 patients with type 1 HRS treated with terlipressin plus albumin in 22 hospitals (84). It represents the results obtained in daily clinical practice, outside pilot or randomized studies.
HRS, hepatorenal syndrome; MCFS, Multicenter French Study.

Ginès and Rodés give a detailed description of the treatment of dilutional hyponatremia and HRS in Chapter 17.

Spontaneous Bacterial Peritonitis

SBP is defined as the infection of a previously sterile ascitic fluid without an apparent intra-abdominal source of infection. The prevalence of SBP in unselected patients with cirrhosis and ascites admitted to a hospital ranges between 10% and 30% (97). Most patients with SBP present with complicated ascites (i.e., ascites plus fever, abdominal pain, diarrhea or ileus, encephalopathy, or renal failure). The incidence of SBP in patients with uncomplicated ascites (i.e., patients admitted only for the treatment of ascites with paracentesis) ranges between 0% and 3.6% (98,99). The diagnosis of SBP is established with a PMN count in ascitic fluid higher than 250 cells/mm^3. In approximately 50% to 60% of the cases, the organism responsible is isolated in ascitic fluid culture or in blood cultures. The remaining cases are considered as a variant of SBP (neutrocytic ascites) and are managed in the same way as those with a positive culture (100). The outcome among patients with cirrhosis and SBP has dramatically improved during the last 20 years. In the last randomized controlled trial comparing cefotaxime versus cefotaxime plus intravenous albumin infusion at the time of diagnosis of infection, the hospital mortality among patients receiving albumin was only of 10% (13). An early diagnosis of SBP, the use of third-generation cephalosporins, and the expansion of plasma volume at the time of diagnosis of infection to prevent the impairment of circulatory function induced by SBP are the most likely reasons for the improvement in SBP prognosis. Mortality associated with SBP is due to the development of a severe impairment of circulatory function leading to multiorgan failure (3,5). Patients with cirrhosis recovering from an episode of SBP should be considered potential candidates for liver transplantation because the survival expectancy after this bacterial infection is very poor.



▪ **Figure 19.18** Pathogenesis of spontaneous bacterial peritonitis.

Pathogenesis

Colonization of the ascitic fluid from an episode of bacteremia is the most accepted hypothesis of the pathogenesis of SBP (Fig. 19.18). Although the passage of microorganisms from the bloodstream to ascitic fluid

has never been documented, it can be assumed that bacteria present in the circulation can easily pass to the ascitic fluid and exchange between these two compartments. Once bacteria have reached the ascitic fluid, the development of SBP depends on the antimicrobial capacity of the ascitic fluid. Patients with a decreased defensive capacity of ascitic fluid develop SBP.

The most common organisms isolated from patients with SBP in cirrhosis are bacteria normally present in the intestinal flora. Gram-negative aerobic bacteria from the family of *Enterobacteriaceae*, and individually *Escherichia coli*, are the most common causative organisms. Nonenterococcal streptococcal organisms cause most of the other cases (66). Several pathogenic mechanisms have been proposed to explain the passage of enteric organisms from the intestinal lumen to the systemic circulation. Bacterial translocation is the process by which enteric bacteria normally present in the gastrointestinal lumen cross the mucosa, colonize the mesenteric lymph nodes, and reach the bloodstream through the intestinal lymphatic circulation. Bacterial translocation could be the consequence of the intestinal bacterial overgrowth that leads to an increase in aerobic gram-negative bacilli in the jejunal flora in cirrhosis and of the possible alteration in gut permeability caused by portal hypertension or by circumstances that decrease mucosal blood flow (e.g., acute hypovolemia or splanchnic vasoconstrictor

drugs). Depression of the hepatic reticuloendothelial system allows free passage of microorganisms from the intestinal lumen to the systemic circulation through the portal vein and prolongs bacteremia. The skin, the urinary tract, and the upper respiratory tract may be the sites through which nonenteric bacteria enter the circulation and cause SBP. This pathogenic mechanism is favored in many cases by diagnostic or therapeutic procedures, which break the natural mucocutaneous barriers. Whatever the source of the bacteria that reach the bloodstream, a bacteremic event is more prolonged and, therefore, can more readily become clinically significant in patients with cirrhosis than in those without this disease because of the marked depression of the reticuloendothelial system associated with cirrhosis.

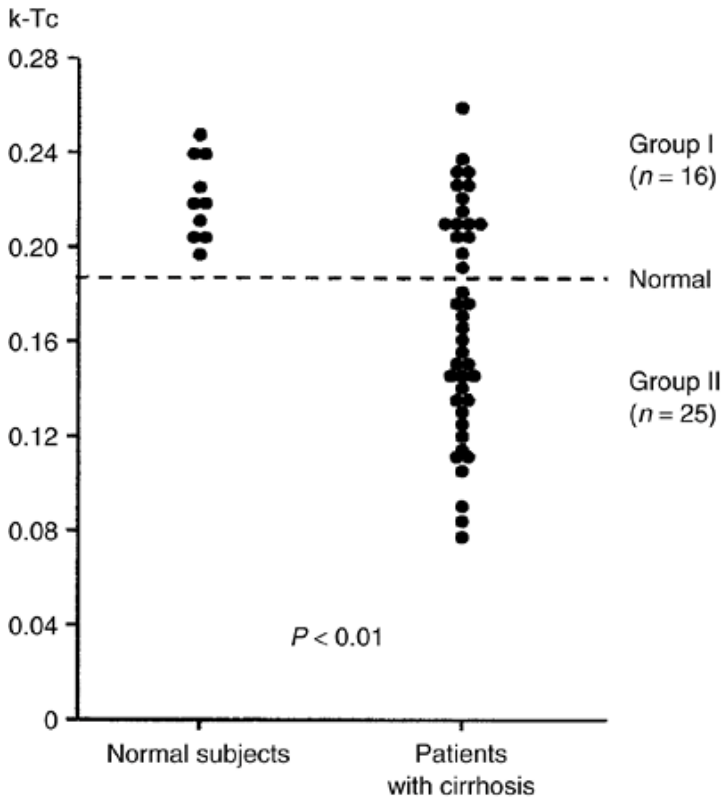
Bacterial translocation

Most studies on bacterial translocation have been performed in rats with cirrhosis induced by carbon tetrachloride. It is shown that in these animals there is an increased passage of bacteria from the intestinal lumen to extraintestinal sites, including regional lymph nodes and the systemic circulation. Causes for bacterial translocation are a disruption of the intestinal permeability barrier, bacterial overgrowth, and a decrease in host immune defenses. The simultaneous presence of intestinal bacterial overgrowth and a severe disturbance in the intestinal barrier seem to be required for bacterial translocation to mesenteric lymph nodes. The alteration in intestinal permeability could be partially caused by portal hypertension that causes marked edema and inflammation in the submucosa of the cecum, thereby favoring bacterial translocation. Changed permeability of the intestinal mucosa also occurs in hemorrhagic shock, sepsis, or injury, or on administration of endotoxin. Results of experiments on portal hypertension have shown that hemorrhagic shock is followed by increased bacterial translocation to mesenteric lymph nodes. This finding suggests that hemorrhagic shock, a not-infrequent event in patients with cirrhosis, can alter the intestinal barrier in these animals. Overgrowth of gram-negative bacteria is found in the jejunal flora of patients with cirrhosis. The intestinal hypomotility in patients with cirrhosis who have sympathetic overactivity may, at least partly, explain this fact. The change in the intestinal flora may increase the chance that aerobic gram-negative bacteria will invade the bloodstream and cause infection of enteric origin in patients with cirrhosis. In these patients, bacterial translocation to mesenteric lymph nodes seems to be related to the presence of ascites and to the degree of hepatic insufficiency because both are markedly increased in Child-Pugh C patients (101).

Depression of activity of the reticuloendothelial system

Although the reticuloendothelial system is widely distributed throughout the body, approximately 90% of this defensive system is in the liver, where Kupffer cells and endothelial sinusoidal cells are the major components. Patients with cirrhosis may have marked depression of the reticuloendothelial system (Fig. 19.19). The risk of acquiring bacteremia and SBP in cirrhosis is directly related to the degree of dysfunction of the reticuloendothelial system (102). Reticuloendothelial cell function in cirrhosis is also a predictor of survival (Fig. 19.20). The mechanism of the impairment of the phagocytic activity of the reticuloendothelial system in cirrhosis is probably multifactorial. It likely includes intrahepatic shunting, which leads to a lack of contact between the reticuloendothelial cells and the blood; impairment of the intrinsic phagocytic

capacity of the reticuloendothelial cells (103,104); and a reduction in the serum opsonic activity, probably as a consequence of a decreased serum concentration of complement and fibronectin. These substances normally stimulate the phagocytosis of microorganisms by enhancing their adhesiveness to the reticuloendothelial cell surface.



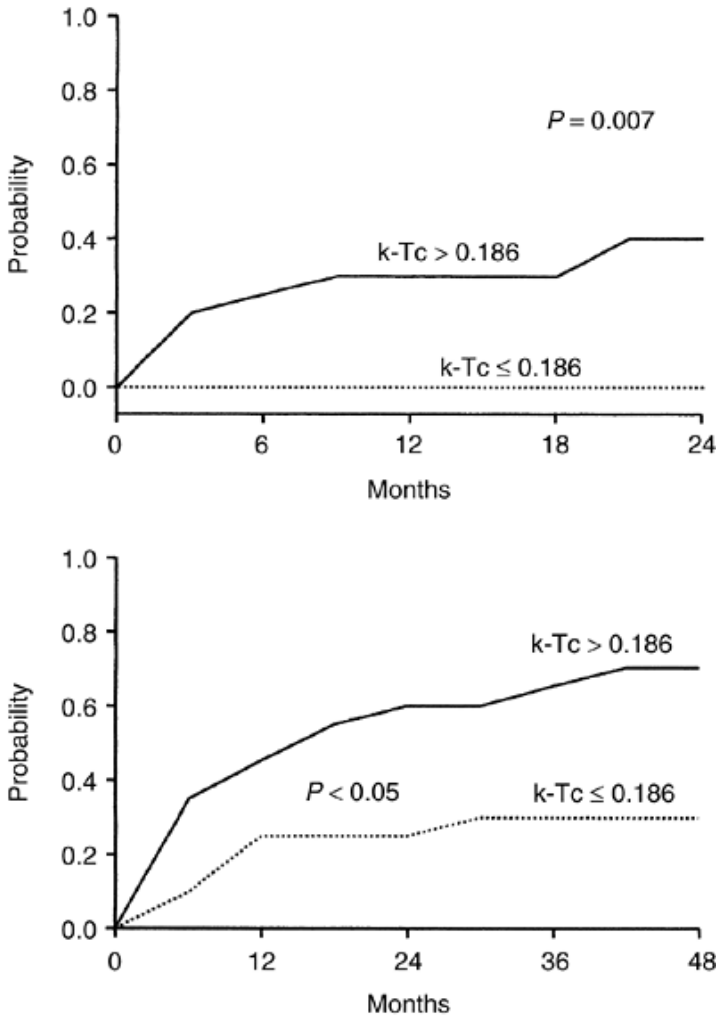
▪ **Figure 19.19** Phagocytic activity of the reticuloendothelial system estimated by the constant elimination rate of albumin labeled with technetium Tc 99m in healthy subjects and in patients with cirrhosis (normal phagocytic activity—group I—and impaired phagocytic activity—group II). (From Rimola A, Soto R, Bury F, et al. Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* 1984;4:53–58, with permission.)

Decreased opsonic activity of the ascitic fluid

The nonspecific antimicrobial capacity of ascitic fluid in cirrhosis varies greatly from patient to patient, and this variability may be involved in the pathogenesis of SBP. There is a highly significant inverse correlation between the opsonic activity of ascitic fluid and the risk of development of SBP among patients admitted to the hospital with ascites (105).

The opsonic activity of ascitic fluid in cirrhosis correlates directly with the total protein level in ascites and with the concentration of defensive substances, such as immunoglobulins, complement, and fibronectin (21,22,106,107,108). It is, therefore, not surprising that the concentration of total protein in ascitic fluid, an

easy measurement in clinical practice, correlates directly with the risk of SBP in cirrhosis with ascites (Fig. 19.21). Patients with protein concentration in ascitic fluid less than 10 g/L contract peritonitis during hospital stay with a significantly higher frequency than do those with a higher protein content in ascites (15% vs. 2%) (22). The cumulative 1-year probability of developing peritonitis is significantly greater in this subgroup of patients with cirrhosis than among those with an ascitic protein concentration greater than 10 g/L (20% vs. 2%) (107). Finally, the probability of the first episode of SBP among patients with cirrhosis and ascites is significantly related to ascitic fluid protein and serum bilirubin levels (108).

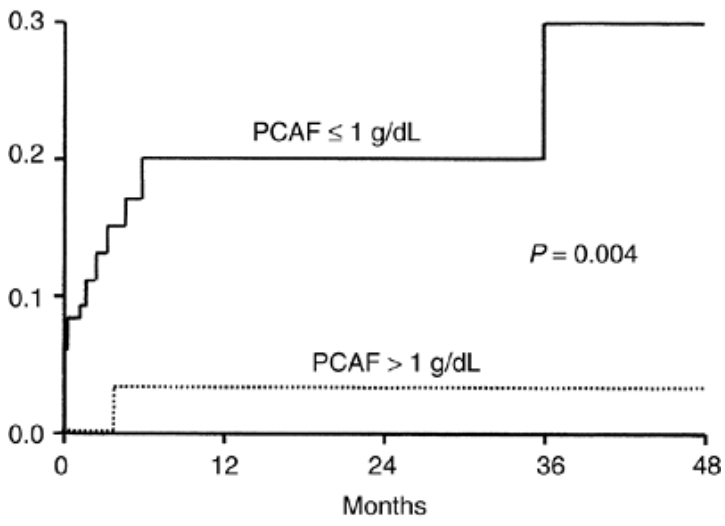


▪ **Figure 19.20** Probability of developing spontaneous bacterial peritonitis or bacteremia (*top*) and probability of survival (*bottom*) according to the phagocytic activity of the reticuloendothelial system (k-Tc ≤ 0.186: Normal phagocytic activity). (From Rimola A, Soto R, Bory F, et al. Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* 1984;4:53–58, with permission.)

Neutrophil leukocyte dysfunction

A high proportion of patients with cirrhosis have altered neutrophil leukocyte function. The most frequent disturbance is a marked reduction of chemotaxis, probably caused by the presence of chemotactic inhibitory substances in the serum. The nature of these substances has not yet been determined. Furthermore, the phagocytic and bacterial killing capacity of neutrophils is reduced in cirrhosis. However, because the type of infection in patients with congenital or acquired abnormalities in neutrophil function (mainly chronic granulomatous diseases and recurrent staphylococcal and fungal infections) is very different from that in patients with cirrhosis, it seems unlikely that leukocyte dysfunction

plays a major role in the susceptibility of patients with cirrhosis to bacterial infection.



▪ **Figure 19.21** Probability of developing the first episode of spontaneous bacterial peritonitis in patients with cirrhosis and ascites according to the protein concentration of the ascitic fluid (PCAF). (From Llach J, Rimola A, Navasa M, et al. Incidence and predictive factors of first episode of spontaneous bacterial peritonitis in cirrhosis: relevance of ascitic fluid protein concentration, *Hepatology* 1992;16:724–727, with permission.)

Iatrogenic factors

Patients with cirrhosis frequently undergo diagnostic or therapeutic maneuvers that can alter the natural defense barriers and, therefore, increase the risk of bacterial infection. Endoscopic sclerotherapy for bleeding esophageal varices, particularly emergency sclerotherapy, is associated with bacteremia in 5% to 30% of cases. Although in some patients sclerotherapy is implicated in the development of serious infections such as purulent meningitis and bacterial peritonitis, bacteremia is usually a transient phenomenon and the use of prophylactic antibiotics is not recommended. The insertion of a TIPS for the management of bleeding esophageal varices is not associated with the development of significant bacterial infections. However, patients with cirrhosis with a peritoneovenous shunt (LeVeen shunt) frequently have infections, particularly bacteremia and peritonitis. In several series the incidence of

bacterial infection after the insertion of a LeVeen shunt for the management of ascites was approximately 20%. The risk of clinically relevant infection with other invasive techniques often performed in these patients, such as diagnostic or therapeutic paracentesis and endoscopy, is low.

Diagnosis

Clinical characteristics

The clinical presentation of SBP depends on the stage at which the infection is diagnosed. When the infection is well developed, most patients have signs or symptoms clearly suggestive of peritoneal infection. However, SBP can be minimally symptomatic or asymptomatic in the initial stages. Abdominal pain and fever are the most characteristic symptoms. Other signs and symptoms, such as alterations in gastrointestinal motility (i.e., vomiting, ileus, diarrhea), hepatic encephalopathy, gastrointestinal bleeding, renal impairment, septic shock, and hypothermia, may be present in many patients. Diagnostic paracentesis should be performed at hospital admission on all patients with cirrhosis and ascites to ascertain the presence of SBP, and on hospitalized patients with ascites whenever they have any of the following: (a) Abdominal pain, vomiting, diarrhea, ileus, or rebound tenderness; (b) systemic signs of infection such as fever, leukocytosis, or septic shock; and (c) hepatic encephalopathy or impairment of renal function.

Laboratory and microbiologic data

The diagnosis of SBP is based on clinical suspicion and on the results of analysis of ascitic fluid. A PMN count of 250 cells/mm^3 in the ascitic fluid is considered the gold standard for the diagnosis of SBP and constitutes an indication to initiate empiric antibiotic treatment. In patients with hemorrhagic ascites a subtraction of one PMN per 250 red blood cells should be made to adjust for the presence of blood in ascites. Leukocyte esterase reagent strips are useful for a rapid bedside diagnosis of SBP. Sensitivity in different series ranged from 83% to 100% and specificity from 89% and 100% (14,15,16,17). Measurement of lactate dehydrogenase concentration, glucose level, and total protein concentration in ascitic fluid is important to establish a differential diagnosis between spontaneous and secondary peritonitis. Secondary peritonitis should be suspected when at least two of the following conditions are present in the ascitic fluid: Glucose levels less than 50 mg/dL, protein concentration greater than 10 g/L, and lactate dehydrogenase concentration greater than normal serum level. Results of Gram stain of a smear of sediment obtained after centrifugation of ascitic fluid are frequently negative for SBP because the concentration of bacteria is usually low (one organism per milliliter or less). Nevertheless, Gram stain may be helpful in identifying intestinal perforation when several types of bacteria are present.

Results of culture of ascitic fluid drawn directly into blood culture bottles (aerobic and anaerobic media) at the bedside are positive in 50% to 80% of cases. Results of blood cultures are also positive in a large proportion of patients with SBP. Other alterations in systemic laboratory values such as leukocytosis, azotemia, and acidosis occur in patients with cirrhosis and SBP.

Treatment

Antibiotic therapy must be started once the diagnosis of SBP is established.

Empiric treatment should cover all potential organisms responsible for SBP without causing adverse effects. At present, third-generation cephalosporins are considered the standard in the management of SBP associated with cirrhosis. Other antibiotics are also effective.

Cefotaxime

Results of the first investigation of the efficacy of cefotaxime in the treatment of patients with SBP were published in 1985 (109). The study was a randomized controlled trial comparing cefotaxime with the combination of ampicillin plus tobramycin in the treatment of a large series of patients with cirrhosis and SBP or other severe bacterial infection. Cefotaxime was more effective in achieving SBP resolution than ampicillin plus tobramycin. Whereas no patient treated with cefotaxime had nephrotoxicity and superinfection, these two adverse effects occurred in more than 10% of the patients treated with ampicillin plus tobramycin. After this study, cefotaxime was considered the first-choice antibiotic in the empiric therapy for SBP in patients with cirrhosis.

Two randomized controlled trials were conducted to assess the optimal dosage of cefotaxime and duration of therapy in the treatment of patients with cirrhosis and SBP (110,111). Ninety patients with SBP were randomized to receive cefotaxime (2 g intravenously every 8 hours) for 10 or 5 days. Resolution of the infection (93.1% vs. 91.2%), recurrence of SBP during hospitalization (11.6% vs. 12.8%), and hospital mortality (32.6% vs. 42.5%) were comparable in the two groups. In a second study 143 patients with SBP were randomized to receive two different dosages of cefotaxime: 2g every 6 hours or 2 g every 12 hours. Rates of SBP resolution (77% vs. 79%) and patient survival (69% vs. 79%) were similar in both groups. Therefore, in patients with SBP, cefotaxime should be used at a dose of 2 g every 12 hours and for a minimum of 5 days.

Other parenteral antibiotics

Ceftriaxone (2 g intravenously every 24 hours) is highly effective in the treatment of SBP. The resolution rate is 90% to 100% and the hospital mortality rate is 30%. Cefonicid (2 g intravenously every 12 hours) is also effective in the treatment of SBP, with a resolution rate of 94% and a hospital mortality rate of 37%. Aztreonam has been evaluated in SBP in a single pilot study. The overall mortality during hospitalization was 62%. Superinfections due to resistant organisms were detected in three cases (19%). These results, together with the fact that aztreonam is only capable of covering approximately 75% of the potential organisms causing SBP, clearly establish that this antibiotic is not adequate for the empiric treatment of patients with cirrhosis and SBP. Finally, two studies have shown that the parenteral administration of amoxicillin–clavulanic acid is effective and safe in the treatment of SBP. The lower cost of this antibiotic regimen in comparison with third-generation cephalosporins is an important advantage.

Oral antibiotics

Patients with SBP may be in relatively good clinical condition and could be treated orally. Two studies have been conducted to assess the effectiveness of oral antibiotics in the management of SBP. In both studies wide-spectrum quinolones were used; these agents are almost completely absorbed after oral administration and rapidly diffuse to the ascitic fluid. In the first study, oral pefloxacin alone (one case) or in combination with other oral antibiotics

(cotrimoxazole, nine cases; amoxicillin, three cases; cefadroxil, one case; and cotrimoxazole–metronidazole, one case) was administered in 15 episodes of SBP. The rate of resolution of infection was 87%. Two patients had superinfections, and the survival rate at the end of hospitalization was 60%. The second study was a randomized controlled trial in patients with nonsevere complications of SBP (i.e., no septic shock, ileus, or serum creatinine concentration >3 mg/dL). Treatment with oral ofloxacin (400 mg every 12 hours) was compared with intravenous administration of cefotaxime (2 g every 6 hours). The study showed a similar rate of infection resolution and patient survival in the two groups. The incidence of superinfection and the length of antibiotic treatment were also similar in two groups. These findings suggested that oral ofloxacin is as effective as intravenous cefotaxime in the management of uncomplicated SBP associated with cirrhosis. Quinolones should not be empirically used in the treatment of patients in whom SBP develops while they are undergoing selective intestinal decontamination with norfloxacin. Third-generation cephalosporins are the best therapeutic option in these patients because they can develop infection from quinolone-resistant bacteria.

Intravenous albumin infusion in spontaneous bacterial peritonitis

For many years the hospital mortality rate associated with SBP (30% to 50%) has been relatively high despite a significant improvement in the rate of resolution of the infection (80% to 90%). Therefore, 20% to 30% of patients with SBP died during hospitalization despite being cured of the infection. Initial studies showed that development of type 1 HRS and not resolution of the infection was the principal predictor

of hospital mortality (112). Subsequent investigations showed that type 1 HRS in patients with SBP occurs in the setting of a rapid deterioration of systemic hemodynamics, with a marked increase in the degree of activity of the renin–angiotensin system and sympathetic nervous system (18,19). SBP-induced circulatory dysfunction develops in patients with marked inflammatory response to the infection (very high ascitic fluid concentration of leukocytes and cytokines) and is associated with an increased production of vasodilatory substances such as nitric oxide and carbon monoxide (113,114). Finally, two recent studies have presented data indicating that impairment of circulatory function in SBP is due to both an accentuation of the arterial vasodilatation already present in these patients and a marked decrease in cardiac output (3,5). These studies also showed that in addition to an impairment of renal perfusion, there is a severe reduction in hepatic blood flow and a marked increase in the intrahepatic resistance to the portal venous flow and portal pressure. The frequent deterioration of hepatic function, the development of hepatic encephalopathy, and the relatively high frequency of variceal bleeding in patients with SBP are probably related to these features.

A recent randomized controlled trial showing that circulatory support with intravenous albumin reduces the incidence of renal impairment and improves hospital survival in SBP has been important in the process of decreasing hospital mortality associated with this infection (13). The study included 126 patients with SBP who were treated with intravenous cefotaxime (63 patients) or with cefotaxime and intravenous albumin (63 patients). Albumin was given at a dose of 1.5 g/kg body weight at the time of diagnosis, followed by 1 g/kg body weight on day 3. Plasma renin activity increased significantly in patients treated with

cefotaxime and decreased in patients receiving cefotaxime plus albumin, indicating that albumin prevents the deterioration of the effective arterial blood volume induced by SBP. Renal impairment developed in 21 patients in the cefotaxime group (33%) and in 6 patients in the cefotaxime plus albumin group (10%). The hospital mortality rate was 29% in the cefotaxime group in comparison with 10% in the cefotaxime plus albumin group. Renal impairment and hospital mortality were extremely low in patients with serum creatinine and/or serum bilirubin levels at time of diagnosis of infection equal to or lower than 1 mg/dL and 4 mg/dL, respectively, in the two therapeutic groups. The results of this study, therefore, indicate that patients with cirrhosis and SBP, particularly those with high serum creatinine or bilirubin levels over 4 mg/dL, should be treated with albumin for volume expansion.

The mechanism by which albumin prevents circulatory dysfunction and type 1 HRS and improves survival has recently been explored in a randomized pilot study comparing the hemodynamic effects of albumin and the synthetic plasma expander hydroxyethyl starch in patients with SBP (115). Albumin but not hydroxyethyl starch improved the effective arterial blood volume, as estimated by the mean arterial pressure and plasma renin activity. This was due both to a greater expansion in central blood volume and an increase in systemic vascular resistance. In patients with SBP, therefore, albumin acts not only as a plasma volume expander but also in the arterial circulation, reducing the degree of arterial vasodilatation. Because the levels of nitric oxide metabolites increased in patients receiving hydroxyethyl starch but not in those treated with albumin and the plasma concentration of the von Willebrand's factor, which is released from the vascular endothelium in parallel with nitric oxide, decreased in patients receiving albumin but not in those treated with hydroxyethyl starch, it was suggested that albumin improves the systemic vascular resistance in SBP by inhibiting the increased activity of nitric oxide synthase by the vascular endothelium.

Predictors of Resolution of Spontaneous Bacterial Peritonitis and of Survival

Several studies have been performed to identify the predictors of resolution of infection and hospital survival in SBP. The results have shown that parameters related to kidney function are the most important predictors of survival. In a retrospective analysis of 213 consecutive episodes of SBP empirically managed with cefotaxime in 185 patients with cirrhosis, multivariate analysis identified 4 out of 51 clinical and laboratory variables obtained at the time of diagnosis of infection (i.e., band neutrophils in white blood cell count, community-acquired versus hospital-acquired SBP, blood urea nitrogen level, and serum aspartate aminotransferase level) as independent predictors of resolution infection and 6 (i.e., blood urea nitrogen level, serum aspartate aminotransferase level, community-acquired vs. hospital-acquired SBP, age, Child-Pugh score, and ileus) as independent predictors of survival (112). In another study of 252 consecutive episodes of SBP, the development of renal impairment after the diagnosis of SBP was the strongest independent predictor of patient mortality in episodes responding to cefotaxime (18). Renal impairment occurred in 83 episodes (33%), and in every instance it fulfilled the criteria of functional renal failure. Renal impairment was progressive in 35 episodes, steady in 27, and transient in 21. The mortality rate was 100% in episodes associated with progressive renal impairment, 31% in episodes associated with steady renal impairment, 5% in episodes with transient renal impairment, and 7% in episodes without renal

impairment. Other independent predictors of mortality

in this series were age, blood urea nitrogen level at diagnosis, isolation of the responsible organism in the ascitic fluid culture, and peak serum bilirubin during antibiotic treatment. Plasma and ascitic fluid cytokine levels also have prognostic value in patients with SBP (19). Renal impairment in SBP occurs in patients with the highest concentration of cytokines in plasma and in ascitic fluid and is associated with marked activation of the renin–angiotensin system. It is likely that renal impairment in SBP occurs as a result of a cytokine-induced impairment of effective arterial blood volume. This is the rationale for the use of plasma expanders in SBP. The recommendation of avoiding diuretics and large-volume paracentesis is also based on this concept.

The development of SBP is associated with poor survival in patients with cirrhosis ascites. The 1-year probability of survival is lower than 40%. Therefore, SBP is considered an important criterion for the indication of liver transplantation.

Prophylaxis

Current indications of intestinal decontamination in SBP prevention are summarized in Table 19.9. Patients with cirrhosis and gastrointestinal hemorrhage are predisposed to develop severe bacterial infections during or immediately after the bleeding episode. Two studies have shown that short-term intestinal decontamination with oral nonabsorbable or poorly absorbable (norfloxacin) antibiotics is effective in preventing bacterial infections and SBP in patients with cirrhosis and gastrointestinal hemorrhage (116,117). The usefulness of systemic administration of prophylactic antibiotic agents to patients with cirrhosis and gastrointestinal hemorrhage was investigated in three controlled studies. In these studies the treated groups received ofloxacin (initially intravenously and then orally) plus amoxicillin–clavulanic acid (before each endoscopy examination), ciprofloxacin plus amoxicillin–clavulanic acid (first intravenously and then orally once the bleeding was controlled), and oral ciprofloxacin (118,119,120,121). The incidence of bacterial infections was significantly lower in the treated groups (10% to 20%) than in the corresponding control groups (45% to 66%). In these studies, the effect of systemic antibiotics on SBP was not specifically studied. However, because improvement in survival in the groups receiving antibiotic prophylaxis was also observed, the use of prophylactic antibiotics in the care of patients with cirrhosis and gastrointestinal hemorrhage, independent of their specific risk of SBP was highly recommended. A meta-analysis including all the aforementioned studies, showed a significant benefit in the subgroup of patients with cirrhosis with and gastrointestinal hemorrhage: 95% of patients in the treated group were free of SBP as opposed to 87% in the control group (121).

Table 19.9. Current Indications and Proposed Duration of Selective Intestinal Decontamination in Cirrhosis

Indications	Duration of prophylaxis
Patients with cirrhosis recovering from a previous episode of SBP (secondary	Indefinitely or until liver transplantation

prophylaxis)	
Patients with cirrhosis and gastrointestinal bleeding	7 d
Patients with cirrhosis and ascites and low ascitic fluid protein levels (≤10 g/L)	During hospitalization (no consensus)

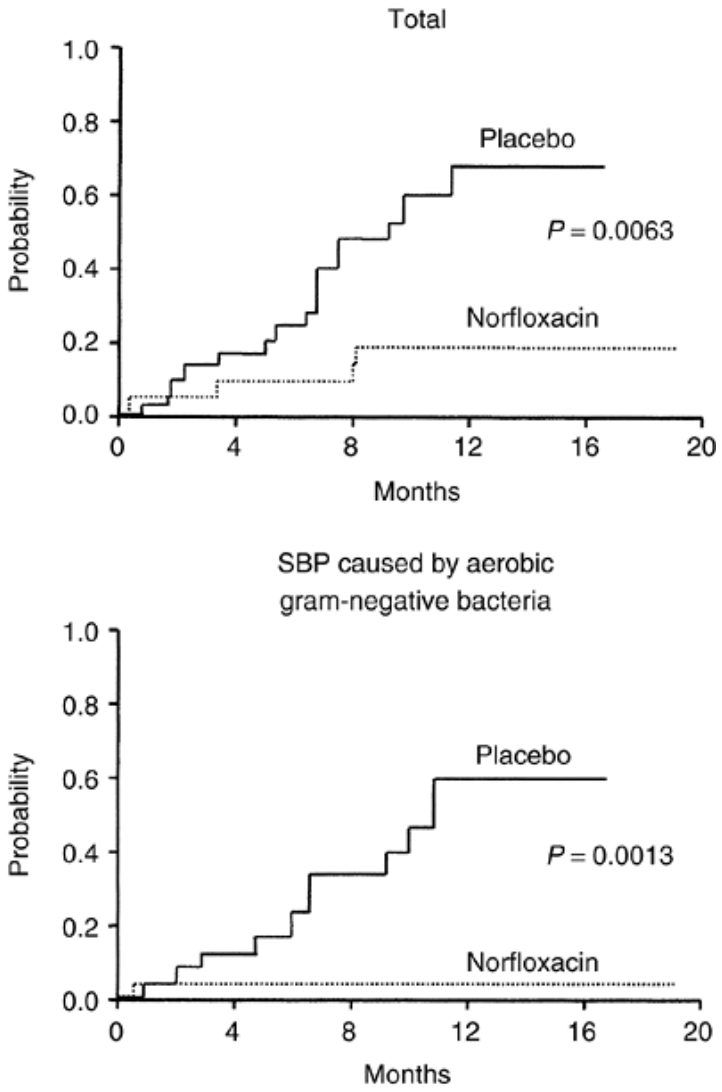
SBP, spontaneous bacterial peritonitis.

Patients with low total protein concentration in ascitic fluid may be a second group of patients with cirrhosis who may benefit from selective intestinal decontamination. Four randomized controlled trials showed that oral antibiotics reduced the incidence of bacterial infections and SBP in this type of patients. In the care of 63 patients admitted to hospital for management of an episode of ascites associated with a low total protein concentration in ascitic fluid (some with previous episodes of SBP), continuous administration of norfloxacin (400 mg/day) throughout the hospitalization decreased the in-hospital incidence of SBP from 22% (control group) to 0% (treated group) (122). In another study in patients with cirrhosis, low ascitic fluid protein concentration, and no previous episodes of SBP, the 6-month incidence of SBP was 0% for the group of patients prophylactically treated with norfloxacin (400 mg/day) and 9% for those treated with placebo (123). A third placebo-controlled study in patients with and without previous episodes of SBP showed that 6-month prophylaxis with ciprofloxacin (750 mg once a week) was effective in reducing the incidence of SBP (4% for the treated group, 22% in the placebo-controlled group) (124). A fourth investigation showed that trimethoprim–sulfamethoxazole (one double-strength tablet 5 days a week) is effective in the prevention of SBP in patients with cirrhosis and ascites (125). The medium follow-up period was only 90 days. However, the incidence of SBP was 26.7% in the control group and 3.3% in the treated group. In this study, patients who were at different levels of risk for SBP (patients with low and high ascitic fluid protein levels and those who did or did not have previous SBP episodes) were included.

Patients recovering from an episode of SBP represent a unique population for assessing the effect of long-term intestinal decontamination in the prophylaxis of SBP because the rate of recurrence of SBP 1 year after treatment may be as high as 70%. In a double-blind placebo-controlled trial including 80 patients with cirrhosis who had recovered from an episode of SBP, the

overall probability of recurrence of SBP after 1 year of follow-up study was 20% in the norfloxacin group and 68% in the placebo group. The probability of SBP caused by aerobic gram-negative bacilli after 1 year of follow-up evaluation was 3% and 60% in the norfloxacin and the placebo group, respectively (Fig. 19.22). Only one patient treated with norfloxacin had side effects related to treatment (oral and esophageal candidiasis) (126). Long-term selective intestinal decontamination, therefore, dramatically decreases the rate of recurrence among patients with SBP. In three economic analyses, investigators calculated that long-

term antibiotic prophylaxis in the care of patients with cirrhosis is associated with reduced cost compared with the "diagnose and treat" strategy. This finding suggests that prophylaxis is cost effective in the treatment of patients at high risk of the development of SBP.

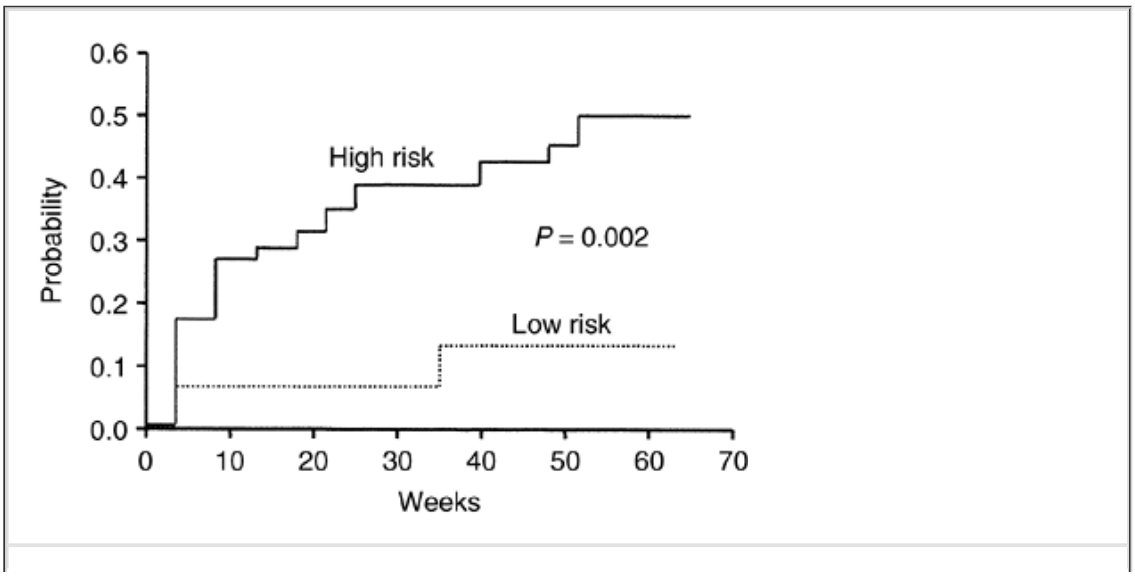


▪ **Figure 19.22** Probability of spontaneous bacterial peritonitis (SBP) recurrence caused by all types of bacteria (*top*) and by gram-negative bacteria alone (*bottom*) in patients receiving norfloxacin prophylaxis or placebo. (From Ginès P, Rimola A, Planas R, et al, Norfloxacin prevents spontaneous bacterial peritonitis recurrence in cirrhosis: results of a double blind, placebo-controlled trial, by Ginès P, *Hepatology* 1990;12:716–724, with permission.)

Antibiotic prophylaxis, therefore, is indicated in the care of patients with cirrhosis who have had a previous episode of SBP because they are at high risk of SBP and because prophylaxis is cost effective. It is also clearly indicated in the care of patients with gastrointestinal hemorrhage independent of the presence of ascites. However, in the care of patients with low protein content in ascitic fluid who have never had SBP, the recommendation is difficult to establish because of the

heterogeneity of the published studies, which also included patients with previous episodes of SBP. Three studies to assess the incidence of and predictive factors for the first episode of SBP in patients with cirrhosis and ascites may be of help in antibiotic prophylaxis. In a series of 127 patients admitted for the treatment of an episode of ascites, the probability of the appearance of the first episode of SBP was 11% at 1 year and 15% at 3 years of follow-up (105). Five variables obtained at admission were significantly associated with a higher risk of SBP during the follow-up (i.e., poor nutritional status, increased serum bilirubin levels, decreased prothrombin activity, increased serum aspartate aminotransferase level, and low protein concentration in the ascitic fluid), but only one (i.e., low protein concentration in the ascitic fluid) had independent predictive value. The 1- and 3-year probabilities of the first episode of SBP in patients with ascitic fluid protein content less than 10 g/L were 20% and 24%, respectively. Among those with ascitic fluid protein content of 10 g/L or greater, the 1- and 3-year probabilities were 0% and 4%, respectively. A clear conclusion from this study is that long-term prophylactic administration of antibiotic is not necessary in the care of patients without previous episodes of SBP and with protein content in ascitic fluid greater than 10 g/L because the risk of development of SBP is negligible. In a similar study performed in 110 patients with cirrhosis consecutively hospitalized for the management of an episode of ascites (107), six variables associated with a higher risk of first appearance of SBP during the follow-up period were identified. These included serum bilirubin level greater than 2.5 mg/dL, prothrombin activity less than 60%, total protein concentration in ascitic fluid less than 10 g/L, serum sodium concentration less than 130 mEq/L, platelet count less than 116,000/mm^3, and serum albumin concentration less than 26 g/L. Only two of these variables (protein concentration in the ascitic fluid and serum bilirubin level) had an independent predictive value (Fig. 19.23). Finally, in one study, patients with cirrhosis, low ascitic fluid protein levels (≤ 10 g/L), and high serum bilirubin levels (>3.2 mg/dL) or low platelet count (<98,000/mm^3) had a 1-year probability of 55% for the development of a first episode of SBP in comparison with 24% among patients with only low ascitic fluid protein levels (108). The results of these studies indicate that routine determination of biochemical values may help identify patients with ascites who are at high risk for developing a first episode of SBP and the

patients, therefore, may benefit from primary antibiotic prophylaxis. This contention, however, requires confirmation by prospective randomized trials.



▪ **Figure 19.23** Probability of the development of the first episode of spontaneous bacterial peritonitis (SBP) in patients with cirrhosis and ascites classified into low-risk and high-risk groups according to the concentration of protein in ascitic fluid and serum bilirubin and/or platelet count. (From Andreu M, Sola R, Sitges-Sarra A, et al. Risk factors for spontaneous bacterial peritonitis, *Gastroenterology* 1993;104:1133–1138, with permission.)

Problem of the Development of Quinolone-Resistant Bacteria

Our concept about infections caused by quinolone-resistant bacteria in patients undergoing long-term prophylactic treatment with norfloxacin has moved from an exceptional event to a relatively frequent phenomenon. Results of initial studies suggested that the risk of development of SBP or other infections caused by quinolone-resistant strains of gram-negative bacilli was low because most recurrences of SBP in patients taking norfloxacin prophylaxis were caused by gram-positive cocci, mainly streptococci (127,128,129) Thereafter, a high incidence of quinolone-resistant strains of *E. coli* in stools of patients with cirrhosis undergoing long-term quinolone prophylaxis was described in several studies. None, however, reported any clinical infection caused by quinolone-resistant *E. coli*. In 1997, the first study involving patients with cirrhosis undergoing long-term norfloxacin prophylaxis for SBP showed a relevant increased incidence of infection, mainly mild urinary infection, caused by gram-negative bacilli resistant to quinolones (90% of *E. coli* isolated were resistant to quinolones) (129). More recently it was shown that 39 out of 106 infections caused by *E. coli* among hospitalized patients with cirrhosis were quinolone-resistant. This finding suggested that the long-term norfloxacin prophylaxis was significantly associated with these types of infections (mainly urinary tract infections). However, the rate of development of SBP caused by quinolone-resistant *E. coli* in decontaminated patients was exceptional (130). Data from the latest study, however, clearly indicates that SBP due to quinolone-resistant bacteria will probably be an important clinical problem in the near future. All cases of bacterial infection diagnosed within a 2-year period among patients with cirrhosis were prospectively evaluated (131). In patients undergoing long-term norfloxacin prophylaxis, quinolone-resistant gram-negative bacilli caused 50% of culture-positive SBP. This finding occurred only among 16% of patients with culture-positive SBP not receiving norfloxacin. Although in this study SBP caused by quinolone-resistant gram-negative bacilli represented only 26% of the cases of culture-positive SBP, quinolone-resistant SBP seems to have emerged as a real problem. This study also showed a high rate of culture-positive SBP caused by trimethoprim–sulfamethoxazole–resistant gram-negative bacteria in patients undergoing long-term treatment with norfloxacin (44%). This finding suggested that this antibiotic is not an alternative to norfloxacin. The effectiveness of norfloxacin in the prevention of SBP is lower than that found in the initial studies. This situation is not surprising because all patients undergoing long-term norfloxacin prophylaxis have quinolone-resistant bacteria in the fecal flora. Despite this observation, the incidence of SBP caused by quinolone-resistant bacteria in patients undergoing long-term norfloxacin prophylaxis is still low. Different explanations have been proposed for this phenomenon, including a

reduction in the intestinal overgrowth or a favorable effect of quinolones on nonspecific immune defenses. Results also suggest that quinolone-resistant bacteria are less invasive than wild-type bacteria.

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Chapter 20
Hepatic Encephalopathy

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Key Concepts

- Hepatic encephalopathy is a neuropsychiatric syndrome that encompasses multiple manifestations resulting from liver failure and/or portosystemic shunting.
- The neurologic abnormalities are potentially reversible with correction of the liver disease and/or the abnormal portal collateral circulation.
- The pathogenesis of hepatic encephalopathy is multifactorial and relates to the exposure of the brain to toxins that arise mostly from the gut.
- Several neurotoxic substances have been implicated in the development of hepatic encephalopathy; ammonia is an important factor in its pathogenesis.
- The most characteristic manifestation is confusional syndrome in patients with cirrhosis, precipitated by a factor that enhances the toxin's effect or load.
- Treatment is based on the identification and correction of the precipitating factor, provision of supportive measures, and the administration of drugs that decrease the production of toxins or antagonize their effects on the brain.
- Management of patients with hepatic encephalopathy, in addition to the assessment of neurologic manifestations, should include the treatment of the underlying liver disease and/or the abnormal portal collateral circulation.

Hepatic encephalopathy (HE) can be defined as a disturbance in central nervous system (CNS) function due to hepatic insufficiency or portosystemic shunting. This vague definition reflects the existence of a spectrum of neurologic manifestations that develop in association with different liver diseases (1). A common link is the potential reversibility of the neurologic manifestations once the abnormality of liver function is corrected, as well as the importance of shunting of blood arising from the portal venous bed into the systemic circulation. HE must be differentiated from the concurrence of neurologic symptoms and liver disease secondary to a common pathogenetic mechanism such as brain and liver damage caused by alcohol or copper (Wilson disease). HE must also be differentiated from neurologic disturbances directly caused by bilirubin accumulation, hypoglycemia, disorders of blood coagulation, or other well-defined abnormalities that are secondary to liver failure.

The nomenclature of HE is confusing. Some terms are used with different meanings by different authors. Some efforts have been made to reach a consensus, especially for the design of clinical trials (2). Despite this limitation, from a clinical perspective HE is generally classified according to the underlying liver disease and the evolution of the neurologic manifestations (Table 20.1). The most frequent liver disease is cirrhosis, usually accompanied by extrahepatic portosystemic shunts (spontaneous or surgical). HE can also be seen in acute liver failure, in which it constitutes a clinical hallmark of the disorder. In rare cases, HE develops in the absence of any sign of parenchymal liver disease and is

caused solely by portosystemic shunting of congenital or surgically induced origin.

Table 20.1. Classification of Hepatic Encephalopathy

Hepatic encephalopathy	Liver disease	Extrahepatic portosystemic shunting	Neurologic manifestations	Specific features
Acute episode				
In cirrhosis	Cirrhosis	Variable	Acute confusional state to coma	Usually precipitated
In acute liver failure	Acute liver failure	Absent	Acute confusional state to coma	Frequently complicated by brain edema and intracranial hypertension
Chronic				
Relapsing	Cirrhosis	Severe	Relapsing episodes of encephalopathy	Usually without precipitating factors
Persistent	Cirrhosis	Severe	Persistent cognitive or motor abnormalities	Generally related to surgically induced shunts
Minimal hepatic	Cirrhosis	Variable	Asymptomatic	Abnormalities revealed by

encephalopathy				neuropsychological or neurophysiologic tests
In patients with portosystemic bypass with no intrinsic liver disease	No signs of parenchymal disease	Large shunts	Relapsing episodes and persistent abnormalities	Rare disorder, secondary to congenital abnormalities or surgical shunts

Along the lines of Ferenci P, Lockwood A, Mullen K, et al. Hepatic encephalopathy–definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002;35:716–721.

The neurologic manifestations of HE are variable. The most distinctive presentation is an acute episode characterized by the sudden onset of an acute confusional state that can evolve into coma. Neuromuscular abnormalities are common, the most characteristic being the presence of asterixis; pyramidal signs may also be present. The term *chronic HE* is reserved for patients who have frequent episodes of encephalopathy or persistent cognitive (e.g., memory loss, confusion, and disorientation) or neuromuscular (e.g., tremor, apraxia, and rarely paraplegia) disturbances. Minimal HE (previously termed *subclinical HE*) corresponds to those neurologic manifestations not obvious at clinical examination but detected with neuropsychological or neurophysiologic tests (3).

Pathogenesis

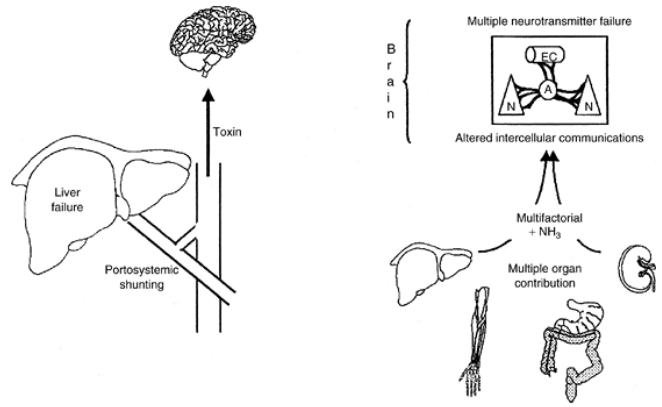
General Aspects

Different hypotheses have been proposed to explain the changes in mental state that occur in HE. Ideally, such a theory should explain the relation between liver abnormalities, neurologic disturbances, and clinical manifestations. However, establishing such relations is difficult, in part because of limitations in the methods available to study brain function in humans in vivo and limitations in knowledge of the neurobiologic basis of behavior. For these reasons, any hypothesis on the pathogenesis of HE should be able to explain the improvement with a specific treatment or account for the mechanism of action of a precipitating factor.

A common pathogenetic notion is that HE is caused by substances that under normal circumstances are efficiently metabolized by the liver, rather than by an insufficient production of substrates that could be essential for neurologic function (Fig. 20.1). In light of this notion, portosystemic shunting plays a critical role because the main impact of this circulatory disturbance is on the concentration of gut-derived substances that are highly cleared by the liver. Studies of crossperfusion in animals with experimental HE and of liver support systems in humans have shown that clearance of toxic substances present in the blood is more important to improve mental function than the synthetic capacity of the support system. In patients with liver disease, these toxic substances reach the systemic circulation through portosystemic shunting or reduced hepatic clearance and produce deleterious effects on brain function. Once the toxic substances are in neural tissues, a large number of neurochemical changes occur that affect multiple neurochemical pathways, each affected to a variable extent.

Historical hypotheses have ranged from single *unifying theories* (4) to the notion of HE as a *multifactorial process* (5). As in other metabolic encephalopathies, general neuronal dysfunction results

in abnormalities of consciousness. However, in contrast to other conditions that affect consciousness (such as hypoglycemia), in which neuronal function is primarily affected, a unique feature of HE is the abnormality of astrocyte morphology and function (See "Abnormalities in the Central Nervous System"). This feature has led to a view that in HE the abnormality in consciousness is the consequence of altered astrocyte–neuronal communications, resulting in changes of multiple neurotransmitter systems (6). Alternative views, such as the γ-aminobutyric acid (GABA) theory, explain the spectrum of HE through the direct effect of a toxin on a key aspect of neurologic function (7). Other paradigms arise from the experimental observation that different toxins (e.g., fatty acids or mercaptans) enhance the negative effects of ammonia on consciousness (*synergistic theory*) (8). More recently, the concept of synergism has been expanded to include the contribution of systemic inflammation to the encephalopathic process (9). Finally, current views also emphasize differing effects of different toxins at various neurologic levels. For example, manganese appears to be involved in parkinsonian manifestations but not in decreasing arousal. In addition, the relative importance of each toxin (See "Putative Toxins") and the site where they cause their main effect (See "Abnormalities in the Central Nervous System") is modulated by different factors (See "Factors that Favor the Effects of Toxins").



▪ **Figure 20.1** A traditional paradigm of the pathogenesis of encephalopathy emphasizing the interplay between liver failure and portosystemic shunting for the availability of toxins in the systemic circulation. A current paradigm includes a multiorgan abnormality in the "periphery." Several factors potentiate the effects of ammonia on the brain, where the presence of multiple neurotransmitter abnormalities can be explained by altered intercellular communications between astrocytes (*A*), neurons (*N*),

and endothelial cells (*EC*).

Putative Toxins

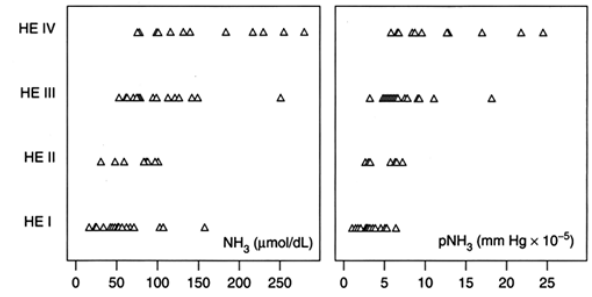
Ammonia

Ammonia has historically been viewed as the most important factor in the genesis of HE. The importance of ammonia in the pathogenesis of HE is highlighted by the following five sets of observations: (a) Ammonia is produced by the gut and a significant amount is of bacterial origin (10), (b) the concentration of ammonia in portal blood is high and a high degree of extraction occurs in the liver (11), (c) concentrations of ammonia are high in the systemic circulation and the cerebrospinal fluid (CSF) of patients with HE (12), (d) precipitating factors cause elevations in the blood level of ammonia or result in the exposure of brain tissue to ammonia (13), (e) treatment strategies of clinical benefit decrease the blood level of ammonia (14).

Ammonia is generated in different tissues by the breakdown of amino acids and other nitrogenous substances (10). Under normal physiologic conditions, ammonia enters the portal circulation from the gastrointestinal tract, where it is derived from colonic bacteria and from the deamidation of glutamine in the small bowel. Traditionally, absorption was viewed as the result of passive diffusion; more recent studies indicate the presence of specific ammonia transporters (15). Regardless of the mechanism of absorption, ammonia reaches high concentration in the portal blood and

undergoes a high first-pass hepatic extraction (80%). In the liver, ammonia is transformed in the periportal hepatocytes into urea (a high-capacity and low-affinity system) and in the centrovenular hepatocytes into glutamine (a low-capacity and high-affinity system). Urea is quantitatively the most important product of ammonia metabolism and elimination. Circulating urea diffuses into the intestine (40%), where it undergoes hydrolysis into ammonia through ureases present in the colonic bacteria. Urinary elimination of nitrogen in the form of urea is a route of ammonia disposal from the organism.

In addition to the intestine and the liver, kidney and muscle contribute to regulate the arterial ammonia level (16). In muscle, ammonia is transformed into glutamine through the action of glutamine synthetase. Experiments in normal volunteers showed that 50% of injected ^{15}N-ammonia is removed by the muscles (17). The ability of the muscle to "fix" appreciable amounts of blood-borne ammonia becomes important for regulating arterial ammonia in case of liver failure and highlights the importance of maintaining an adequate muscular mass by patients with HE. It is generally accepted that at rest skeletal muscle is an ammonia-consuming organ. However, during moderate to heavy exercise, the muscle releases ammonia (18). The kidneys generate ammonia from the deamination of glutamine, a step involved in the regulation of arterial and urinary pH. A small fraction of renal ammonia is released into the systemic circulation; urinary ammonia excretion may be affected by dehydration and increases in conditions of hyperammonemia (19). Notwithstanding the role of peripheral organs, the main factors resulting in the increase in blood levels of ammonia in liver failure are a decrease in the capability of the liver to generate urea and glutamine and the bypass of first-pass hepatic metabolism through portosystemic shunts.



▪ **Figure 20.2** Relationship between venous levels of ammonia and clinical stage of hepatic encephalopathy (HE)—NH_3 (*left graph*, $r = 0.69$, $P < 0.001$) or pNH_3 (*right graph*, $r = 0.79$, $P < 0.001$). (From Kramer L, Tribl B, Gendo A, et al. Partial pressure of ammonia versus ammonia in hepatic encephalopathy. *Hepatology* 2000;31:30–34.)

Patients with HE have an increased diffusion of ammonia into the brain (12), although recent studies using sophisticated positron emission tomography techniques have questioned this tenet (20). Variations in the passage of ammonia across the blood–brain barrier may explain the poor relationship between the level of arterial ammonia and the degree of HE, which nonetheless can be seen when large groups of patients are compared (Fig. 20.2; (21)). Glutamine in brain tissue, which is the product of ammonia metabolism and can be estimated by ^{1}H-magnetic resonance spectroscopy, and glutamine level in CSF is more related to HE than the blood level of ammonia (22).

Ammonia has many deleterious effects on brain function and affects multiple neurotransmitter systems (Table 20.2). However, the clinical manifestations of pure ammonia intoxication differ from the usual manifestations of HE. Patients with urea cycle disorders have symptoms at much higher levels of blood ammonia than those with liver failure. They may also exhibit mental retardation, seizures, and agitation, which are not common in HE. Brain edema leading to intracranial hypertension, a common feature of acute ammonia intoxication, is not clinically relevant in patients with cirrhosis and HE. Additional studies are required to determine whether the differences between these two situations relate to the presence of additional toxins, rate of exposure of the brain, activation of compensatory mechanisms, or other time-dependent factors.

γ-Aminobutyric acid agonists

Several lines of evidence support the presence of activated GABAergic tone in HE (23). One of the postulated mechanisms for this effect is the increased availability of agonist ligands of the GABA receptor complex, a

key inhibitory neurotransmitter in the brain. The term *natural benzodiazepines* has been coined for a group of substances of nonpharmacologic origin that bind to the benzodiazepine site of the GABA receptor, where they can act as an agonist or antagonist. These substances, which are poorly characterized from a chemical and functional perspective, have been reported to be present in a variety of human tissues in normal conditions and to purportedly accumulate in the brain of patients with HE (24). It has been

proposed that natural benzodiazepines with agonistic effects on the GABA receptor induce a decrease of consciousness in HE. However, not all the benzodiazepine ligands found in HE have agonistic effects on the GABA receptor (e.g., diazepam-binding inhibitor). Furthermore, alternative routes of activation of GABA neurotransmission may be present. These include direct and indirect effects of ammonia on the affinity of GABA receptors to its natural ligand (7). Ammonia may also lead to an increased density of peripheral-type benzodiazepine receptors (PTBRs) present in astrocytic mitochondria and whose activation results in the synthesis of neurosteroids, powerful ligands of neuronal GABA receptors (25,26).

Table 20.2. Effects of Ammonia on Nervous Tissue

Effects	Possible consequences
Blocking of chloride channels	Impairment of postsynaptic inhibition
Increase in the transport of neutral amino acids and cerebral tryptophan	Interaction with serotonin-related neurotransmission
Decrease in the activity of α-ketoglutarate dehydrogenase	Decrease in cerebral energy metabolism
Enhancement of the synthesis of neurosteroids	Agonistic effects on GABA neurotransmission
Modulation of GABA receptor	Agonistic effects on GABA neurotransmission
Upregulation of peripheral benzodiazepine receptors	Agonistic effects on GABA neurotransmission
Downregulation of glutamatergic synaptic uptake	Interaction with glutamate-related neurotransmission
Increase in brain glutamine	Interaction with glutamatergic neurotransmission Brain edema
Increase in nitric oxide synthesis	Interaction with glutamatergic neurotransmission

GABA, γ-aminobutyric acid.
Butterworth RF. The neurobiology of hepatic encephalopathy. *Semin Liver Dis* 1996;16:235–244.

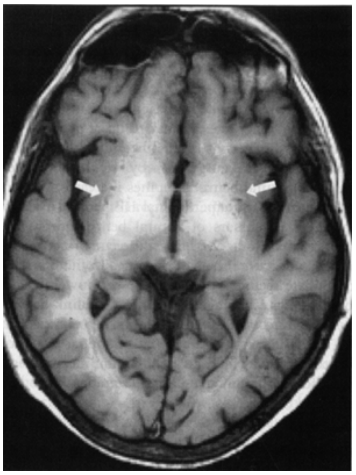
Several arguments have been proposed in favor of a role for natural benzodiazepines in HE. The most relevant is the observation of an improvement of mental state after the administration of flumazenil (a benzodiazepine receptor antagonist) in some patients with advanced stages of HE who have not consumed benzodiazepines of pharmacologic source (27). However, the beneficial effects of flumazenil, usually mild and transient, are only seen in a subgroup of patients. One of the main limitations of this theory is the lack of an explanation of the mechanism by which the concentration of natural benzodiazepines increases in HE. A study in rats with experimental HE showed the generation of precursors of natural benzodiazepines in the intestinal flora (28). These precursors are transformed into natural benzodiazepines in the brain and accumulate secondary to liver failure. However, additional studies in humans to confirm the link between intestinal flora, liver function, natural benzodiazepines, and HE are lacking. An alternative source of natural benzodiazepines could be hemoglobin; metabolites of hemoglobin that mimic benzodiazepines have been described (29).

Manganese

Manganese is probably involved in the development of parkinsonian manifestations in HE, but its role in other neurologic manifestations is uncertain (30). The concentration of manganese is elevated in the plasma of patients with cirrhosis and in the brain of patients who die with HE. Hypermanganesemia is the result of portosystemic shunting and a reduction in biliary excretion (31). Patients with cirrhosis typically exhibit a hyperintense signal in the globus pallidum (Fig. 20.3) that has been attributed to the preferential accumulation of manganese in the basal ganglia. However, some studies have failed to show a good association between the intensity of the signal in basal ganglia and neurologic manifestations of HE (32). Nevertheless, its similarities to the clinical and radiologic features of manganese intoxication suggest that the increase in manganese level in cirrhosis causes the extrapyramidal signs of chronic HE through mechanisms that impair dopaminergic neurotransmission. The effect of manganese removal on the neurologic signs and symptoms of chronic HE has not been evaluated.

Other compounds

A group of potentially neurotoxic compounds of colonic origin has been postulated to affect neurologic function. These include GABA (23), mercaptans, and short-chain fatty acids (8). The concentration of these compounds have been found to be elevated in the plasma of patients and in experimental models of HE, but controversial results and lack of confirmatory data do not support a primary role in HE (Table 20.3).



▪ **Figure 20.3** T1-weighted magnetic resonance image of the brain of a patient with cirrhosis. The patient exhibits a symmetrical hyperintensity of the globus pallidus (*arrows*).

Patients with liver failure show an increase in aromatic amino acid levels (e.g., tyrosine, phenylalanine, or tryptophan) and a decrease in branched-chain amino acid levels (e.g., valine, leucine, or isoleucine). It was proposed that this imbalance would enhance the passage of aromatic amino acids through a neutral amino acid carrier into the brain in exchange for glutamine generated from ammonia detoxification (4). The excess of aromatic amino acids would then be channeled in the brain into the synthesis of *false neurotransmitters* (e.g., octopamine, phenylethanolamine) and serotonin, an inhibitory neurotransmitter. However, this hypothesis, which was the basis for the treatment of HE with branched-chain amino acids, has not been supported by the result of in vivo and postmortem studies. If there is any beneficial effect of the therapy for HE with branched-chain amino acids, it may be through alternative mechanisms (See "Principles of Treatment").

Table 20.3. Other Compounds that Have Been Involved in the Pathogenesis of Hepatic Encephalopathy

Substance	Pros	Cons
GABA	Increase in plasma levels (33) Disturbance in GABAergic neurotransmission (23)	GABA not increased in central nervous system (34)
Short-chain fatty acids	Synergistic effects with ammonia (8) Lactulose decreases the genesis (35)	Lack of correlation between plasma levels and grade of HE (36)
Mercaptans	Synergistic effects with ammonia (37)	Plasma levels found in HE are not neurotoxic (38) Lack of correlation between plasma levels and grade of HE (39)
Aromatic amino acids	Increase in plasma levels (40)	Lack of correlation between plasma levels and grade of HE (41) Normal blood–brain barrier permeability to amino acids (42) False neurotransmitters not found in human brain (43)

GABA, γ-aminobutyric acid; HE, hepatic encephalopathy.

Abnormalities in the Central Nervous System

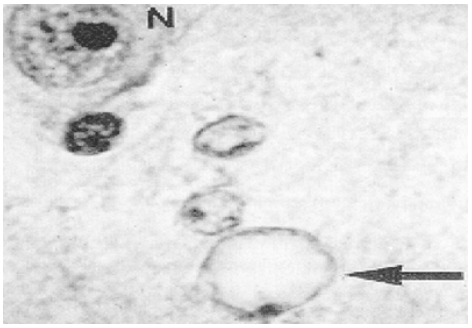
Astrocytes

Experimental and pathologic evidence points at astrocytes as the prominent cells affected in HE (44). No significant or consistent morphologic changes have been identified in neurons or other cells of the CNS. The distinctive morphologic alteration is the Alzheimer type II astrocytic change, which is characterized by a cell with enlarged, pale nuclei with peripheral margination of chromatin and often prominent nucleoli (Fig. 20.4). Results of microscopic studies of specimens from humans and of experimental preparations suggest that the astrocytic changes can be explained by the existence of cellular swelling.

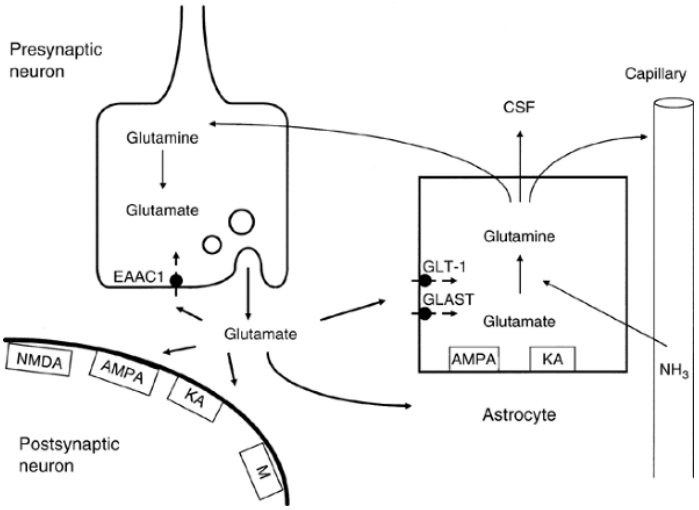
Astrocytes occupy one third of the volume of the cerebral cortex. Their foot processes surround brain capillaries, where they contribute to blood–brain barrier function, and neurons. This anatomic organization forms a syncytium, where critical metabolic supportive functions involved in the maintenance and regulation of the extracellular microenvironment, such as uptake of ions and neurotransmitters, influence neuronal excitability and neurotransmission. A specific

astrocyte function is the detoxification of ammonia through the amidation of glutamate to glutamine. An increase in intracellular

osmolality as a result of glutamine accumulation underlies the genesis of astrocytic swelling in HE (46). These findings have led to the proposal of HE being the clinical manifestation of a gliopathy, in which neuronal dysfunction develops as the result of astrocytic abnormalities (6). Several mechanisms by which abnormal glial cells could influence neuronal function have been postulated:



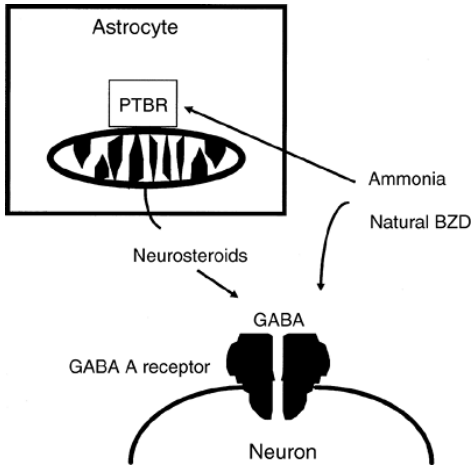
▪ **Figure 20.4** Alzheimer type II astrocyte showing nuclear enlargement and clearing (*arrow*), with the chromatin displaced to the periphery. Two adjacent relatively normal astrocytes are also present. A nearby neuron (*N*) is normal (45). (From Norenberg MD. Hepatic encephalopathy. In: Kettenmann H, Ransom BR, eds. *Neuroglia*. New York: Oxford University Press, 1995:950–963, with permission.)



▪ **Figure 20.5** Abnormalities of glutamate neurotransmission in hepatic encephalopathy. Glutamate released from the presynaptic neuron is again taken up into the perineuronal astrocyte through the glutamate transporter (GLT-1) and glutamate–aspartate transporter (GLAST). Glutamate receptors are expressed in neurons (*N*-methyl-D-aspartate [NMDA], α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA], kainic acid [KA], or metabotropic subtypes [M]) and astrocytes (AMPA, KA). CSF, cerebrospinal fluid; EAAC1, excitatory amino acid carrier 1. (Adapted from Butterworth RF. The neurobiology of hepatic encephalopathy. *Semin Liver Dis* 1996;16:235–244.)

1. Interaction with glutamate reuptake (Fig. 20.5) (47). In experimental models, glial reuptake of the glutamate released from presynaptic neurons is likely to be decreased. Downregulation of glutamate transporters located in the plasma membrane of astrocytes (i.e., glutamate transporter-1, glutamate-aspartate transporter) can be reproduced in astrocyte cultures exposed to ammonia. A decrease in reuptake will result in an increase in brain extracellular glutamate levels, with subsequent effects on glutamatergic neurotransmission.
2. Activation of the PTBRs (Fig. 20.6) (44). An increase in the number of PTBRs has been observed in the brain of patients who died with HE and can be reproduced experimentally after administration of ammonia. Activation of PTBRs by different ligands, such as ammonia and diazepam-binding inhibitor, results in an increase in the synthesis of neurosteroids (e.g., pregnenolone, dehydroepiandrosterone), powerful ligands of the neuronal GABA A receptor, thereby affecting GABAergic neurotransmission.
3. Metabolic consequences of cellular swelling (6). The cellular hydration state regulates cell function and gene expression. Swelling of astrocytes

activates extracellular regulated protein kinases, elevates calcium concentration, and affects multiple ion channels and amino acid transport. Oxidative stress can be detected in vitro and in vivo (48). All these abnormalities may affect the ability of astrocytes to efficiently uptake or release extracellular ions and neurotransmitters, secondarily affecting glial–neuronal communication.



▪ **Figure 20.6** Abnormalities of γ-aminobutyric acid (GABA) neurotransmission in hepatic encephalopathy. The activation of "peripheral-type" benzodiazepine receptor (PTBR) by ammonia enhances the synthesis of neurosteroids in the mitochondria of astrocytes. Neurosteroids and ammonia may modulate the GABA A receptor and thus contribute to hepatic encephalopathy. BZD, benzodiazepine. (Adapted from Butterworth RF. The neurobiology of hepatic encephalopathy. *Semin Liver Dis* 1996;16:235–244.)

Table 20.4. Main Abnormalities of Neurotransmission Described in Hepatic Encephalopathy and Their Possible Consequences

System	Findings	Possible consequences
Glutamate	Decrease in total brain glutamate level (50) Increase in extracellular glutamate level (49) Decrease in glutamate transporter levels (51) Decrease in glutamate receptor levels (49)	Impaired mental function Brain edema Convulsions
GABA	Signs of increased GABAergic tone (23) Positive response to flumazenil (27) Increase in benzodiazepine receptor ligand levels (23) Activation of peripheral-type benzodiazepine receptor (44) Increase in neurosteroid levels (44)	Decrease of consciousness
Serotonin	Increase in the metabolism of serotonin (52)	Behavioral abnormalities
Dopamine	Decrease of dopamine receptor levels (53) Increase in degradation of dopa (54) Improvement of extrapyramidal signs with dopa (55)	Extrapyramidal manifestations
Opioid	Increase in the sensitivity to morphine (56) Increase in endogenous opioids (57)	Behavioral abnormalities
Histamine	Increase in histamine receptors (58)	Circadian rhythm abnormalities
Nitric Oxide	Decrease in cerebellar cyclic guanosine monophosphate (59)	Learning impairment

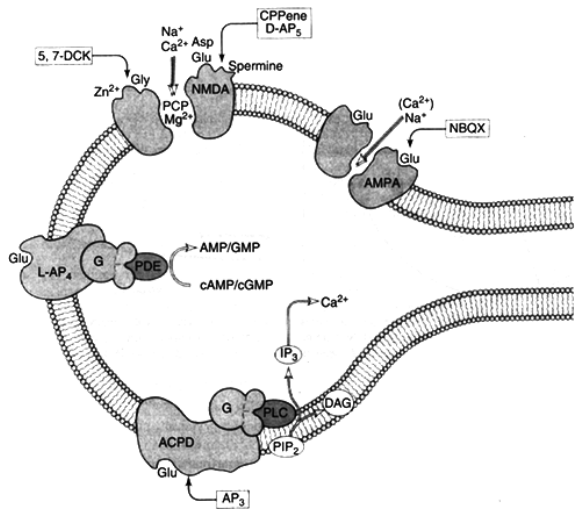
GABA, γ-aminobutyric acid.

Neurotransmitter systems

HE, as other forms of metabolic encephalopathy, appears to occur as a result of abnormalities in neurotransmission (5). This hypothesis is supported by its potential reversibility and by the lack of neuronal damage. Multiple abnormalities of neurotransmitter systems have been described (Table 20.4). Glutamate neurotransmission is clearly disturbed in animal models of HE (49), where it appears to have a role in the pathogenesis of HE (Fig. 20.7). However, supportive human data arise mostly from autopsied samples (50), and pharmacologic manipulation of glutamatergic neurotransmission has not been attempted.

The improvement of neurologic manifestations after the administration of a drug that interacts with an individual transmitter system is an important argument to support a pathogenic role for that system. The first attempt to normalize the abnormalities of neurotransmission arose from the false neurotransmitter hypothesis. The notion was to restore the abnormalities in the profile of plasma amino acids and the transport of amino acids across the blood–brain barrier by administering branched-chain amino acids. Subsequent therapeutic attempts have been focused on the brain itself and include stimulation of dopaminergic transmission with

bromocriptine or levodopa (55) and blockage of GABA-inhibitory neurotransmission with flumazenil (27). The results have not been remarkable, highlighting the complexity of a paradigm in which several neurotransmitter systems are simultaneously affected. Still, these attempts indicate it may be possible to treat HE using drugs that act in the brain in addition to measures that decrease the plasma level of a putative toxin.



▪ **Figure 20.7** Molecular views of four types of glutamate receptors. Two heteromeric receptors are shown, the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, and two metabotropic receptors, the L-AP_4 and trans-(±)-1-amino-cyclopentane-1,3-dicarboxylate (ACPD) receptors. Competitive antagonists of each receptor are boxed. AMP, adenosine monophosphate; cAMP, cyclic adenosine monophosphate; GMP, guanosine monophosphate; cGMP, cyclic guanosine monophosphate; PLC, phospholipase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; PCP, purine-cytosine permease; DAG, diacyl glycerol; IP_3, inositol triphosphate; Glu, glutamine; PDE, phosphodiesterase; DCK, 5,7-di-chlorokynurenic acid; Gly, glycine (60). (From Dingledine R, McBain CJ. Excitatory amino acids. In: Siegel GJ, Agranoff BW, Albers RW, et al. eds. *Basic neurochemistry*, 5th ed. New York: Raven Press, 1994:367–387, with permission.)

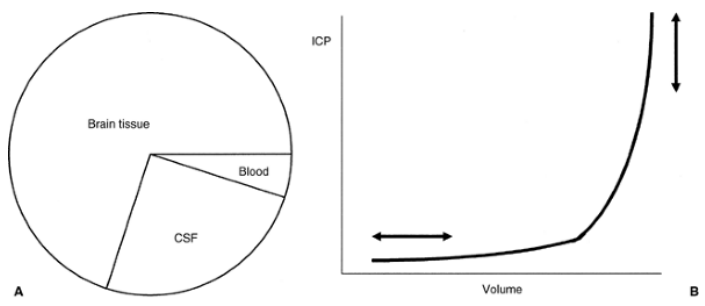
Energy abnormalities

The brain is the tissue with the highest energy requirements of the body and depends entirely on the process of glycolysis and respiration within its own cells to fulfill its energy demands. In HE in humans, a decrease in consumption of oxygen and glucose is accompanied by a parallel decrease in cerebral blood flow (61). These energy abnormalities are not homogeneous across the brain, with basal ganglia exhibiting a different pattern from the cortex (20). Some studies with humans have shown focal reductions of glucose utilization that are related to specific neurologic manifestations (62). However, the findings cannot separate whether the decrease is the cause or the consequence of the encephalopathy. The current interpretation is that, as in other metabolic encephalopathies, the decrease in energy consumption is secondary to the decrease in demand. As observed in some patients with high cerebral blood flow, especially among those with fulminant hepatic failure, an increase in supply does not improve the mental state (63). Ammonia may impair glycolysis because it inhibits α-ketoglutarate dehydrogenase, the rate-limiting enzyme of the tricarboxylic acid cycle (22). However, the histologic features are different from those observed in hypoglycemia or hypoxia. In experimental preparations, energy deficits are only observed after prolonged periods of coma. Results of magnetic resonance spectroscopy performed on humans suggest that there are no significant deficits in the generation of high-energy compounds in the brain (64).

Brain edema

Brain edema is a complication of fulminant hepatic failure, which can progress to intracranial hypertension and death (Fig. 20.8). Brain edema has been frequently regarded as a distinct entity, dissociated from the neurologic features of HE. However, several lines of evidence relate brain edema to HE (46). Although intracranial

hypertension is a common problem in patients with fulminant hepatic failure in coma, the development of high intracranial pressure (ICP) in patients with cirrhosis in deep coma is only occasionally documented (65).



▪ **Figure 20.8 A:** A pie chart separating the three compartments in the brain according to their relative volume: Brain tissue (70%), cerebrospinal fluid (CSF) (25%) and blood volume (5%). **B:** In an early stage of increase in brain volume, large

changes in volume result in small changes in intracranial pressure (ICP); at a later stage, brain compliance is reduced, and small changes in volume cause large changes in pressure.

One important limitation is the assessment of brain edema in these circumstances. Standard neuroimaging techniques are insensitive to detect increases in brain water even when intracranial hypertension is already present. MRI provides multiple indirect evidences of an increase in brain water (66). Magnetization transfer imaging is a technique based on the transfer of magnetization between free protons in water and bound protons associated with macromolecules that allows an estimation of the amount of free water through the calculation of the magnetization transfer ratio (MTR). Brain edema causes a decrease in MTR, a result that is well documented in cirrhosis (67). In addition, the development of low-grade brain edema in cirrhosis is supported by diffusion weighted imaging (68) and magnetic resonance spectroscopy (69). The corticospinal tract, which corresponds to the first neuron of the voluntary motor pathway, appears more vulnerable to edema and functional impairment (70). The parallel improvement of magnetic resonance abnormalities and neurophysiologic disturbances after liver transplantation supports the hypothesis that astrocytic edema may cause secondary neuronal dysfunction (6).

Brain edema appears to originate from the accumulation of glutamine, the product of ammonia metabolism in astrocytes (46). The osmotic effects of an acute increase in glutamine concentration appear to overcome the compensatory capacity of astrocytes, cells that are swollen in neuropathologic preparations. Brain edema has been described in all situations of acute hyperammonemia and has been associated with plasma levels of ammonia in fulminant hepatic failure (71). In the experimental setting, brain swelling secondary to ammonia infusion can be prevented with the administration of an inhibitor of the synthesis of glutamine. Other factors, such as hyponatremia, may enhance the effects of ammonia on brain swelling (72). In fulminant hepatic failure, an additional factor that plays an important role in the development of intracranial hypertension is the presence of abnormalities of cerebral circulation. Cerebral vasodilatation and loss of autoregulation are characteristic findings in fulminant hepatic failure (63). The mechanism that causes the abnormalities of cerebral circulation has not been fully elucidated. They appear to arise from a signal generated in the brain. Indeed, measures that decrease cerebral vasodilatation are of clinical benefit for patients with severe intracranial hypertension (73). In addition to differences in the cerebral circulation and in the rate of exposure of the brain to ammonia, patients with cirrhosis may activate compensatory mechanisms that counteract osmotic changes in the brain (46). Those with hyponatremia are at higher risk for the development of intracranial hypertension.

Factors that Favor the Effects of Toxins

Precipitating factors

Several factors are known to precipitate an episode of HE in stable patients with cirrhosis (Table 20.5). They exert their effects through an increase in the generation of putative toxins, impairment in liver function (resulting in enhanced portosystemic shunting and

larger delivery of toxins to the brain), or enhancement of the effects of the toxins on the CNS. In some cases the mechanisms that explain the action of the precipitating factor seem obvious (i.e., worsening liver function in acute hepatitis). In other cases, there are multiple factors acting as coprecipitants.

Table 20.5. Precipitating Factors for Hepatic Encephalopathy

Precipitating factor	Possible effects	Mechanism of action	Associated coprecipitant
Sepsis	Increase in blood ammonia level Enhancement of the effects of putative toxins on the CNS	Protein catabolism Activation of cytokines	Azotemia Arterial hypotension
Gastrointestinal bleeding	Impairment in liver function Increase in blood ammonia level	Hepatic hypoperfusion Nitrogen load Disturbances of plasma amino acids	Infection Anemia Arterial hypotension
Hypokalemia	Increase in blood ammonia level	Ammonia generation	—
Azotemia	Increase in blood ammonia level	Ammonia generation	—
Dehydration	Increase in blood ammonia	Hepatic hypoperfusion	Hypokalemia Azotemia
Diuretics	Increase in blood ammonia	Hypokalemia Azotemia Dehydration	—
Acute hepatitis	Impairment in liver function Enhancement of effects on the CNS	Liver injury Activation of cytokines	
Surgery	Impairment in liver function	Hepatic hypoperfusion	Anesthetics
Constipation	Increase in blood ammonia	Ammonia generation by enteric flora	—

Large protein intake	Increase in blood ammonia	Nitrogen load	—
Psychoactive drugs	Enhancement of effects on the CNS	Activation of inhibitory neurotransmission	—

CNS, central nervous system.

Infection and inflammation have been postulated to play an important synergistic role in the pathogenesis of HE (9). Possible mechanisms by which such effects may be mediated include activation of vagal afferents at the site of inflammation, binding of cytokines and/or inflammatory cells to receptors in cerebral endothelial cells with subsequent transduction of signals into brain, and direct access of cytokines into brain tissue to sites lacking blood–brain barrier (such as the circumventricular organs). Cytokines may increase blood–brain barrier permeability to ammonia, resulting in the generation of intracerebral mediators, such as nitric oxide and prostanoids, and cause astrocytic swelling (6,74). A systemic infection will also impair renal function, increasing circulatory urea levels, with subsequent colonic generation of ammonia through urease-containing bacteria. Treatment of infection has been shown to have a direct impact on neuropsychological function in patients with cirrhosis (75).

Portosystemic shunts

Portosystemic shunting allows the access of gut-derived toxins into the systemic circulation. There are three different types of portosystemic shunts: (a) Congenital shunts without significant liver disease, (b) large spontaneous shunts in cirrhosis, and (c) procedural shunts.

Congenital shunts are rare conditions that connect the portal with the systemic circulation and may cause neurologic manifestations compatible with HE without abnormalities in the liver parenchyma (76). Different morphologic types have been described. They may be single or multiple and be located intrahepatic or extrahepatic. Congenital shunts may be associated with hypoplastic portal vein branches or even the absence of portal vein (patent ductus venosus or Abernethy malformation). Associated abnormalities in the portal branches may lead to some degree of parenchymal atrophy. Clinical manifestations present very early in life or in the sixth or seventh decades, suggesting an age-dependent sensitivity of the CNS to develop HE.

Large portosystemic shunts may develop in some patients with cirrhosis and favor the development of persistent HE (77). These spontaneous shunts can decrease portal pressure, and the patients seldom have significant portal hypertension. These shunts may have different extrahepatic locations. Among the different shunts, large splenorenal shunts are those more commonly associated with chronic HE because they lead to marked portal flow steal (78).

Procedural shunts are secondary to transjugular intrahepatic portosystemic shunt (TIPS) or other surgical intervention (79). The frequency of postshunt encephalopathy depends on the type of shunt and the susceptibility of the individual. Approximately, one third of patients subjected to a TIPS procedure will

develop encephalopathy. Nonselective portosystemic shunts (i.e., portocaval, mesocaval) produce more encephalopathy than do selective shunts (i.e., distal splenorenal) in patients with nonalcoholic cirrhosis. However, selectivity of splenorenal shunts is lost in the long term. Elderly patients and those with poor liver function are at higher risk for postshunt encephalopathy. However, there is no hepatic functional test that confidently identifies individuals who will develop HE. Closure of TIPS is associated with improvement of HE (80).

Increased brain susceptibility

Patients with cirrhosis prone to HE have an increased susceptibility to the effects of different psychoactive drugs, such as morphine, antidepressants, or benzodiazepines. This increased susceptibility is not explained simply by pharmacokinetic changes induced by liver failure (81). Hypersensitivity to psychoactive drugs may be mediated by changes in neurotransmission secondary to the disease of the liver, such as underlying abnormalities in benzodiazepine receptors. Additional factors could be the presence of abnormalities at the level of the blood–brain barrier and/or cerebral blood flow. General derangements of blood–brain barrier permeability do not appear to be present in HE (46). However, selective increments in permeability may occur, as has been shown for ammonia in patients with minimal HE (12). Comorbid conditions, which are common in patients with cirrhosis, such as alcoholism or dilutional hyponatremia, as well as advanced age, may facilitate the development of HE because of their direct effects on brain function.

Clinical Features

The Acute Episode of Encephalopathy

An acute episode of HE is characterized by the development of an acute confusional syndrome that includes impaired mental state, neuromuscular abnormalities, fetor hepaticus, and hyperventilation (1). Variability is an important feature; the clinical manifestations may fluctuate rapidly and oscillate from mild confusion to deep coma. The onset is usually abrupt; HE develops over hours to days. Most patients do not have significant neurologic manifestations before the onset of the acute episode of HE, unless they had persistent HE. The evolution of an acute episode of HE tends to parallel the course of liver function or the removal of the precipitating factor. Prolonged episodes of HE occur among patients with terminal liver failure. Patients usually recover from HE without major neurologic deficits and are able to return to previous activities.

Impairment of consciousness initially manifests as subtle changes of personality or disturbances in the circadian rhythm of sleep and wakefulness (i.e., insomnia during the night, somnolence during the day). As HE progresses, the manifestations include inappropriate behavior, disorientation, confusion, slurred speech, stupor, and coma. Some patients may experience nausea and vomiting, especially if there is rapid evolution into coma.

Asterixis is a characteristic feature of HE that represents the failure to actively maintain posture or position (1). Asterixis is caused by abnormal function of diencephalic motor centers that regulate the tone of the agonist and antagonist muscles normally involved in maintaining posture (82). The classic method of eliciting asterixis is by dorsiflexion of the patient's hand, with the arms outstretched and fingers separated. The postural lapse that occurs consists of a series of rapid, involuntary, flexion–extension movements of the wrist. Asterixis may be observed during any sustained posture: Tongue protrusion, dorsiflexion of the foot, or fist

clenching. Asterixis is not exclusive to HE and can occur in other metabolic or structural encephalopathies (e.g., renal failure, hypercapnia, stroke affecting basal ganglia). Asterixis does not occur in early or advanced HE. In coma, asterixis disappears, but the patient may exhibit signs of pyramidal involvement, such as exaggerated deep tendon reflexes, hypertonia, or extensor plantar responses. Transient decerebrate posturing and abnormal ocular movements may occur in deep coma.

Fetor hepaticus is a peculiar pungent odor of the breath that is often regarded as a component of HE. This odor is attributed to dimethylsulfide, a volatile sulfur compound, that can be identified in the breath and serum of patients with cirrhosis (39). The presence of fetor hepaticus is not constant; patients with cirrhosis but not HE can have this condition. Hyperventilation is also frequent, especially among patients with advanced HE, and has been interpreted as a compensatory mechanism that decreases the entrance of ammonia into the brain through a decrease in arterial pH. It has also been related to elevated levels of estrogens and progestogens (83).

The Patient with Chronic Encephalopathy

Chronic encephalopathy encompasses two different situations: (a) The patient with relapsing episodes of HE and (b) the patient with persistent neurologic manifestations. This differentiation highlights the more prominent clinical presentation, but in practice both situations are difficult to separate. Some patients initially

have relapsing episodes and later have persistent symptoms. A patient with purely relapsing HE or purely persistent HE is rare. Furthermore, symptoms tend to fluctuate after the institution of therapeutic measures or the occurrence of precipitating events.

Relapsing episodes may be due to precipitating factors, but in most cases are spontaneous or related to the discontinuation of medication. A history of constipation is commonly elicited. The course of the acute episode does not differ from the one previously described, except a tendency for an abrupt onset and resolution. Between episodes, the patient can be perfectly alert and not show any sign of cognitive dysfunction. However, a careful neurologic examination and neuropsychological tests may reveal abnormalities. Mild parkinsonian signs, characterized mostly by bradykinesia without tremor (84), are probably the most common manifestation between episodes.

Persistent HE refers to those manifestations that do not reverse despite adequate treatment. In most patients with cirrhosis and prior episodes of acute HE and advanced liver failure, a careful neurologic examination will reveal multiple mental and motor abnormalities. Most of these abnormalities are subtle, such as increased muscle tone, reduced mental or motor speed, dysarthria, hypomimia, lack of attention, or apraxia. Psychometric tests may be helpful in describing and quantifying the degree of impaired mental function.

Persistent HE is considered severe when it impairs daily activities. The most characteristic manifestations of severe chronic HE are dementia, severe parkinsonism, or myelopathy in combination with other manifestations of neurologic involvement (e.g., ataxia, dysarthria, gait abnormalities, or tremor). This clinical picture is seldom seen nowadays because of the availability of liver transplantation and the limited number of patients who undergo surgical portosystemic shunts. Patients with *hepatic dementia* tend to have fluctuating symptoms with periods of improvement and a subcortical pattern. The initial manifestations are attentional deficits, visuopractic abnormalities, dysarthria, and apraxia. Those with *hepatic parkinsonism* may resemble Parkinson's disease, except for a symmetrical presentation and lack of significant tremor. *Hepatic myelopathy* (85) is characterized by a progressive spastic paraparesis accompanied by hyper-reflexia and extensor plantar responses. Only a few patients have sensory symptoms or incontinence. The pathogenetic mechanisms of these complications are obscure. They have associated neuronal loss—in case of dementia—and demyelination along the pyramidal tract—in case of myelopathy. Although these lesions are difficult to reverse, there are descriptions of improvements after liver transplantation (86), a challenge to the notion of irreversibility. The term *hepatocerebral degeneration* has been occasionally used to describe such patients. However, this is a neuropathologic diagnosis applied to those patients whose brains exhibit substantial and irreversible loss of gray matter in the cortex and basal ganglia. It is preferable not to use it to describe the clinical picture.

The Brain in Fulminant Hepatic Failure

The clinical picture of HE in acute liver failure parallels that of an acute episode of HE: An acute confusional syndrome that evolves into coma. However, in acute liver failure, brain edema leading to intracranial hypertension and abnormalities of brain perfusion is critical (87).

Brain edema does not result in clinical manifestations unless intracranial hypertension is present because the displacement of brain tissue is the factor that results in neurologic symptoms. Intracranial hypertension may manifest as decerebrate rigidity, myoclonus, seizures, mydriasis, bradycardia, or arterial hypertension (Cushing's reflex). However, the diagnosis of intracranial hypertension based on clinical signs is unreliable because they can be absent with pressures as high as 60 mm Hg (88) and are difficult to monitor because these patients are intubated and paralyzed when they are in coma.

A major consequence of intracranial hypertension is the effect on cerebral perfusion. The maintenance of cerebral blood flow is critical to ensure an adequate supply of oxygen. The driving force in maintaining a stable blood flow is the cerebral perfusion pressure, the arithmetical difference between mean arterial pressure and ICP. When cerebral perfusion pressure is less than 40 mm Hg, structural tissue damage from brain ischemia may ensue. In spite of low cerebral blood flow, an occasional patient may recover from this situation without irreversible brain damage. Another consequence of intracranial hypertension is the mechanical compression of neighboring structures. The increase in pressure causes displacement of brain tissue, resulting in herniation and direct compression of the temporal lobe or the cerebellum. Brain stem compression can result in sudden respiratory arrest and circulatory collapse.

Minimal Hepatic Encephalopathy

Minimal HE, also referred by the terms *latent* or *subclinical*, is a mild dysfunction of brain function that cannot be detected by standard clinical examination (3,89). This label was originally applied to a group of individuals who performed abnormally on psychometric tests but had essentially normal findings on clinical examination. Psychometric tests are more sensitive than clinical observation, as shown in other neuropsychiatric diseases, such as dementia.

Other techniques (e.g., electroencephalogram [EEG]), evoked potentials, or neuroimaging) that are more sensitive than clinical examination to reveal neurologic impairment have also shown a stage of minimal dysfunction, which is understood as part of a continuous disorder that has several levels of severity, minimal HE being the mildest expression of HE. This interpretation is supported by the observation of amelioration of minimal HE after using the same therapeutic measures as those used against *overt* HE (90) and the relationship between minimal HE, ammonia levels, and liver function (91).

The diagnosis of minimal HE is arbitrary and can be performed with neuropsychological or neurophysiologic tests. The most characteristic deficits are in motor and attentional skills (92). Learning impairment, which has also been described in experimental models (59), appears to be the consequence of attention deficits (93). The depth of the psychometric and the clinical examination necessary to diagnose minimal HE is not defined. The frequency of the diagnosis is variable (30% to 84% of patients), depending

on the characteristics of the population being studied and the extent of the psychometric evaluation. Some attempts have been made to develop practical tools on the basis of the design of short batteries of neuropsychological tests, such as the Psychometric Hepatic Encephalopathy Score (PHES) (94). However, these batteries have not been fully standardized and their use is still investigational. Critical flicker frequency, a neurophysiologic tool, has been proposed as a practical test to assess low-grade encephalopathy (95).

The importance of establishing the diagnosis of minimal HE is unknown. Some studies have highlighted that minimal HE may have an adverse impact on the ability to perform daily activities and on health-related quality of life (96). However, many subjects are able to compensate for these deficits (89). From a practical point of view, a psychometric evaluation may be adequate in those individuals whose occupations demand attentional and motor abilities. A report of impaired driving in patients with minimal HE (97) suggests the need to develop a therapeutic program for such individuals. Benefits of treatment, as assessed by monitoring the neuropsychological response, should be weighed against secondary side effects. There are no data on the effects of therapy on health-related quality of life. Patients with cirrhosis and minimal HE have a clear tendency to develop overt HE (98). Whether the institution of preventive measures would decrease the risk of the progression to overt encephalopathy has not been evaluated. The presence of minimal HE indicates worse prognosis, especially if associated with high levels of blood ammonia after the administration of glutamine (99). For this reason, liver transplantation should be considered in patients with minimal HE.

Methods for the Assessment of Hepatic Encephalopathy

Grading Hepatic Encephalopathy

Grading of HE is necessary to assess the evolution of the condition and the response to the effects of therapy. Several methods are based on clinical findings or the combination of neurophysiologic and neuropsychological tests, but the simplest grading of HE is based on clinical findings. The West Haven index is widely used (13). It is based on changes in consciousness, intellectual function, and behavior (Table 20.6). The Glasgow Coma Scale offers a system that monitors consciousness according to simple and more objective parameters. This scale was initially developed for traumatic coma but has gained widespread use for all forms of coma. It is probably more reliable than the West Haven criteria but has the limitation that it is less sensitive in quantifying the mildest forms of HE and is better suited for advanced HE.

The portosystemic encephalopathy (PSE) index has been used in many studies to assess the effects of therapeutic measures. This index combines the assessment for mental state, arterial ammonia level, EEG, the number connection test, and estimation of the degree of asterixis. An arbitrary weight of 3 is assigned to the mental state and the other parameters are weighted. Concerns have been raised about the arbitrary scoring system, the inclusion of ammonia (a putative toxin), the feasibility of an arterial puncture, and the assessment of the number connection test in the evaluation of advanced HE. It is generally considered that blood levels of ammonia, although separating groups according to mean values (21), show wide dispersion in individual values and are not useful to predict the severity of HE and monitor the response to therapy (100). A

consensus has been reached indicating that the PSE index is not adequate for clinical follow-up and is not recommended for clinical trials.

Table 20.6. Grading Scale of Hepatic Encephalopathy Based on Change in Mental Status

Grade	Neurologic manifestations
0	No alteration in consciousness, intellectual function, personality, or behavior
1	Trivial lack of awareness, euphoria or anxiety; short attention span
2	Lethargy, disorientation, personality change, inappropriate behavior
3	Somnolence to semistupor, confusion; responds to noxious stimuli
4	Coma, no response to noxious stimuli

From Conn H. & Bircher J. with mild modifications Conn HO. The hepatic encephalopathies. In: Conn HO, Bircher J, eds. *Hepatic encephalopathy. Syndromes and therapies*. Bloomington, IL: Medi-Ed Press, 1994:1–12.

Neuropsychological Tests

The main role of neuropsychological tests is the diagnosis of minimal HE and the assessment of cognitive function in patients with persistent HE. On the basis of the most frequently found abnormalities, several psychometric tests have been proposed to be the most adequate for diagnosing HE (89,92). Neuropsychological tests can be affected by multiple factors. It is important that the neuropsychological assessment takes into consideration these factors. Patients with clear signs of decreased arousal cannot undergo testing. Care is needed to control for comorbidities, visual impairment, or cultural barriers. The test should be adapted to the cultural characteristics of the population being evaluated. Nomograms to compare the results should take into consideration age and, ideally, the degree of education. The patient undergoing testing should be seated in a quiet room with sufficient light. An important limitation of the neuropsychological tests is the practice effect in follow-up evaluation. Results of psychometric tests are affected by learning. Use of parallel versions can lessen this effect, but only few tests have well-standardized versions.

Some short batteries specifically developed for HE may be useful for the detection of abnormal cognition. However, they do not substitute a formal neuropsychological evaluation performed by an experienced neuropsychologist. The *PHES* is a battery of tests specifically developed for the diagnosis of minimal HE (94). Similar to the Mini-Mental State Examination for dementia, PHES can be useful for screening minimal HE. The PHES combines five paper–pencil tests (i.e., line tracing tests, digit symbol test, serial dotting test, number connection test A, and number connection test B) that examine motor speed and accuracy, visual perception, visual–spatial orientation, visual construction, concentration, attention, and, to a lesser extent, memory. The results of the battery are scored according to normograms from a group of healthy controls. Each one of the test scores 0 points when it falls in the ±1 standard deviation (SD) range. A test that falls in the range more than 1 SD is scored +1 point and for less than -1 SD, -2 SD, -3 SD range, the tests are scored with -1, -2, or -3 points, respectively. Thereby, the subjects could achieve between +6 and -18

points. A pathologic test result (diagnosis of minimal HE) is set at -4 points.

Neurophysiologic Tests

A large number of different neurophysiologic tests have been proposed for the diagnosis and quantification of HE. Reports for and against the specificity of electrophysiologic changes have been published (101,102). These tests are most useful in documenting cerebral dysfunction in difficult cases and possibly in monitoring response to therapy. They have the advantage of not being influenced by learning effects. Therefore, they may be better suited for assessing effects of treatment than neuropsychological tests, especially for advanced stages of HE. For minimal–mild HE, neurophysiologic tests do not give information about behavioral consequences, in contrast to the insight provided by neuropsychological tests.

The standard EEG shows slowing of the frequency from the normal 8 to 13 Hz to the delta range below 4 Hz. The change usually commences in the frontal or central regions and progresses posteriorly. High-voltage, low-frequency (1.5 to 3 Hz) waves with triphasic appearance have been considered characteristic of HE. However, they have been described in a variety of forms of metabolic encephalopathy and are not specific for HE. Several stages of evolution of EEG changes have been described in HE, and a fair correlation with clinical stages and ammonia levels has been observed. The simplest EEG assessment is to grade the degree of abnormality of the conventional tracing. Computer-assisted frequency analysis of the EEG includes evaluation of the mean dominant frequency and the power of a particular EEG rhythm. Minor changes in the dominant frequency occurs in patients with minimal HE (91).

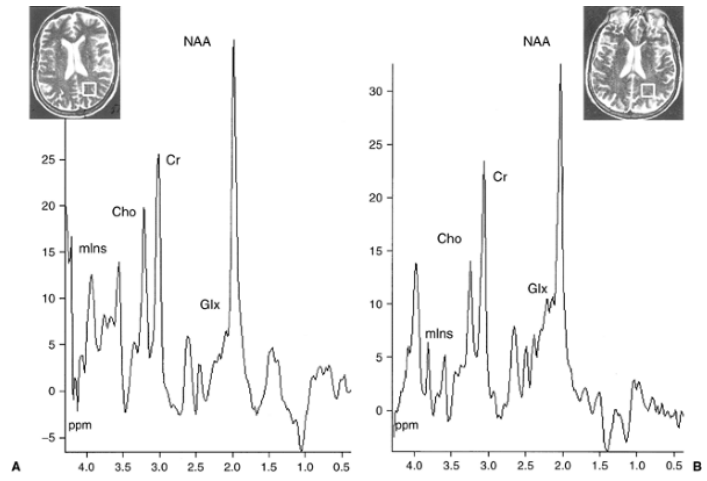
Evoked responses are externally recorded potentials reflecting discharges through neuronal networks after exposure to specific stimuli (102). Depending on the type of stimulus and the pathway analyzed, they could be visual, somatosensory, or acoustic-evoked potentials. Event-related potentials using different stimuli represent an endogenously task-related cortical response reflecting the neural pathway involved in awareness, learning, and decision-making processes. Event-related potentials, such as the P300-evoked potential, requires patient cooperation and well-trained operators. Evoked potentials and event-related potentials are considered more sensitive than the conventional EEG for the diagnosis of mild forms of HE. They may be useful for assessing the presence of minimal or mild HE in patients with cirrhosis who have memory loss or other mental symptoms.

Neuroimaging

At autopsy, the brains of patients with cirrhosis who have died from HE do not show major anatomic abnormalities, except for various degrees of atrophy. Therefore, neuroimaging studies that exclusively assess the morphologic structure of the brain, such as computed tomography (CT) scan, do not detect specific abnormalities in HE. Brain atrophy, which is depicted with CT scan, is more common in patients with

long-standing cirrhosis and chronic HE (103). However, brain atrophy is not a specific abnormality of HE and may be related to factors other than HE (e.g., alcoholism, age, or comorbid conditions). Furthermore, as in other neurodegenerative diseases, brain atrophy is not associated with neuropsychological performance (104). Conventional neuroimaging techniques are insensitive to the detection of brain swelling that may develop in some patients with cirrhosis and frequently complicates HE in acute liver failure (105). No studies have been able to find a neuroimaging correlate of hepatocerebral degeneration (cortical laminar necrosis and polymicrocavitation at the corticomedullary junctions and in the striatum).

Magnetic resonance imaging (MRI) and spectroscopy allow the acquisition of data on cerebral metabolic function that are otherwise not available (64). Proton MRI shows a typical pallidal hyperintensity on T1-weighted images (Fig. 20.3). This abnormality is most frequently seen in patients with cirrhosis and severe liver failure or long-standing portosystemic shunts and is absent or only minimally present in patients with well-compensated cirrhosis and unimpaired neuropsychiatric function. It can be also present in patients with congenital shunts or portal thrombosis and normal liver function (106). No direct correlation between the magnitude of pallidal hyperintensity and the grade of HE have been found, but some studies have related pallidal hyperintensity to parkinsonian manifestations (107). Because of radiologic similarities to manganese intoxication, it has been proposed that pallidal hyperintensity is the consequence of the preferential deposition of manganese in the basal ganglia. The deposition of manganese in brain tissue would be secondary to portosystemic shunting and might be involved in the parkinsonian symptoms found in persistent HE.



▪ **Figure 20.9** Proton magnetic resonance spectroscopy. White matter spectrum representation from a healthy control **(A)** and a cirrhotic patient with chronic HE and mild neuropsychological impairment at the time of the study **(B)**. The most significant peaks correspond to *myo*inositol (mIns, 3.55 ppm), choline (Cho, 3.2 ppm), creatine (Cr, 3.0 ppm), glutamine/glutamate (Glx, 2.15 to 2.50 ppm), and *N*-acetyl aspartate (NAA, 2.0 ppm). The spectrum of the patient with cirrhosis shows a marked decrease in mIns and an increase in Glx. (From Cordoba J, Hinojosa C, Sanpedro F, et al. Usefulness of magnetic resonance spectroscopy for diagnosis of hepatic encephalopathy in a patient with relapsing confusional syndrome. *Dig Dis Sci* 2001;46:2451–2455, with permission.)

Proton magnetic resonance spectroscopy allows the assessment of several brain metabolites (e.g., glutamine, glutamate, *myo*inositol) that may be related to the pathogenesis of HE. The level of glutamine, the product of ammonia metabolism in astrocytes, is characteristically increased in brain tissue. Although glutamine is considered neuronally inactive, changes in its concentration may affect neuronal–astrocytic trafficking and affect glutamate neurotransmission (44). The concentration of

glutamine in CSF, an indicator of its level in brain tissue, has been correlated to the stage of HE. Unfortunately, the standard available systems in which magnetic fields of 1.5 T are used do not allow a separation between the peaks of glutamine (moderately high increase in HE) and glutamate (mild decrease in HE). *Myo*inositol has an important role in osmotic regulation in astrocytes. The decrease in brain *myo*inositol content found by spectroscopy has been corroborated in experimental preparations and has been attributed to a compensatory response to the increase in intracellular osmolality caused by the increased concentration of glutamine (46). Although the technique is insensitive to mild changes in the concentration of metabolites, the abnormalities found with spectroscopy (Fig. 20.9) have been related to neuropsychological impairment and liver function (69). The role of proton magnetic resonance in the diagnosis of HE has not been investigated. Nevertheless, a completely normal study in a patient suspected to suffer from HE is a strong argument against this diagnosis.

Regional distribution of radionuclides in the brain has been used to study cerebral blood flow, oxygen and glucose consumption, neurotransmitter utilization, and availability of neuronal receptors. The results of some of these studies are controversial (64). Although they may help in understanding the pathogenesis of HE, radionuclide studies are not adequate for diagnostic purposes.

Principles of Treatment

HE is a manifestation of severe liver failure; its treatment cannot be separated from the treatment of liver failure. Nevertheless, several measures specifically designed to manage HE appear to be beneficial. The effects have not been evaluated by well-designed randomized clinical trials including a large number of patients. Study design is especially complex in this condition because the clinical course of HE tends to resolve and relapse spontaneously in many cases. The concurrence of other disorders (e.g., anemia, electrolytic disturbances, fever, severe infection, or alcoholic injury) are confounding factors that complicates the assessment of the neurologic manifestations. For these reasons, almost all modalities of therapy have been criticized. In fact reexamination of the results obtained in available trials have questioned the evidence base for current therapies (108). Despite these limitations, critical reappraisal of available data and the clinical experience render it possible to devise a rational approach to the management of HE.

Nutrition

Classically, the recommendation for patients with HE has been to restrict dietary protein intake. The extent of the restriction will depend on the degree of HE, being more marked for severe HE. Many investigators have recommended withholding all protein intake and subsequently increasing intake in increments to maximal clinical tolerance (109). This recommendation has been criticized (110). Only one randomized study has investigated the effects of protein restriction on the outcome of HE (111). In this study 30 patients admitted for an episode of HE received progressive amounts of proteins (from 0 to 1.2 g/kg per day) or normal protein amounts (1.2 g/kg per day) from the beginning. The diet was administered through nasogastric tube for 2 weeks and HE was assessed, blinded for the group of treatment. The main result of the study was that there were no differences in the outcome of HE; the normal protein diet was metabolically more adequate. Therefore, restriction of proteins in the diet does not appear to have any beneficial effect for episodic HE.

Protracted nitrogen restriction may be harmful, as witnessed in patients with acute alcoholic hepatitis (112). Severe malnutrition, which is common among patients with cirrhosis, is associated with a poor short-term prognosis. Although avoiding intake of large amounts of protein may be advantageous for reducing the levels of toxins involved in HE, restriction may worsen liver function and increase the risk of death. A positive nitrogenous balance may improve encephalopathy by promoting hepatic regeneration and increasing the capacity of the muscle to detoxify ammonia. For these reasons the current recommendation is to avoid restrictions of dietary protein (110).

Improvement in nutritional status in patients with cirrhosis and encephalopathy is difficult. A high protein intake (1.2 g/kg per day) may be necessary to maintain nitrogen balance. However, in a classical study (109) the investigators related the intake of increasing quantities of protein to the precipitation of HE. Modifying the composition of the diet and increasing its calorie/nitrogen ratio may improve tolerance to protein. At isonitrogenous levels, vegetable and dairy products cause less encephalopathy than does meat (113). Differences in amino acid composition and in the ratio of carbohydrates to total protein could explain these effects. A high calorie to nitrogen ratio, which is characteristic of casein-based and vegetable-based diets, reduces gluconeogenesis and has anabolic effects on the utilization of dietary proteins. The benefits of vegetable-based diets are also related to the presence of nonabsorbable fiber that is metabolized by colonic bacteria. Fiber increases the elimination of nitrogen products in stool, probably through a similar mechanism to that of nonabsorbable disaccharides.

Branched-chain amino acids were promoted as a means of correcting the imbalance in the plasma amino acid profile, which was thought to be involved in the pathogenesis of HE. However, clinical trials using

branched-chain amino acids have not shown major beneficial effects for episodes of HE and only mild effects for chronic HE (Table 20.7). Branched-chain amino acids do not show significant effects on survival. Critical reviews of the published studies highlight the inadequate design of most studies. Considerations of cost-effectiveness indicate that branched-chain amino acids should not be used outside clinical trials (114). They show anticatabolic effects in patients with chronic liver diseases, probably because of their ability to serve as an energy substitute for muscle and because of their actions on muscle protein synthesis and degradation. This nutritional effect may result in an improvement of liver function and a better clinical outcome, as shown in a multicenter trial performed in Italy that included patients with advanced cirrhosis, most of them without prior HE (115).

Table 20.7. Overview of Randomized Controlled Trials of Branched-Chain Amino Acids for Hepatic Encephalopathy

Treatment	Control	Type of hepatic encephalopathy	Trial	*N*	Design	Duration	Effects on encephalopathy
BCAA IV + glucose 20%	Lactulose + glucose 20%	Acute	Rossi-Fanelli et al. 1982 (116)	34	Multicenter	2–4 d	=
BCAA IV + glucose 50%	Lactulose + glucose 50%	Acute	Vilstrup et al. 1990	65	Multicenter double-	<16 d	=

+ lactulose			(117)		blind		
BCAA IV. + glucose 50% + lipid 20%	Glucose 5% + glucose 50% + lipid 20%	Acute	Wahren et al. 1983 (118)	50	Multicenter double-blind	<5 d	=
BCAA IV + glucose 25%	Neomycin + glucose 25%	Acute	Cerra et al. 1985 (119)	75	Multicenter double-blind	14 d	+
BCAA IV + hypertonic glucose	Neomycin + glucose 50%	Acute	Strauss et al. 1986 (120)	29	Two-center	5 d	=
BCAA IV + glucose 30% + lactulose	Lactulose + glucose 30%	Acute	Fiaccadori et al. 1985 (121)	48	Multicenter	7 d	+
BCAA IV + glucose 30% + lipid 20%	Conventional amino acid mixture + glucose 30% + lipid 20%	Acute	Michel et al. 1985 (122)	70	Multicenter double-blind	5 d	=
BCAA oral 20-g increments + diet 20 g protein	Dietary protein 20-g increments + diet 20 g protein	Chronic	Horst et al. 1984 (109)	26	Multicenter double-blind	30 d	+
BCAA oral 0.3 g/kg + diet 0.5–0.8 g/kg protein	Lactulose + diet 0.5–0.8 g/kg protein	Chronic	Riggio et al. 1984 (123)	90	Single-center	90 d	=
BCAA 0.24 g/kg + usual diet	Casein 0.18 g/kg + usual diet	Chronic	Marchesini et al. 1990 (124)	64	Multicenter double-blind	90 d	+
BCAA oral 0.25 g/kg + diet 1 g/kg protein	Casein 0.25 g/kg + diet 1 g/kg	Latent	Egberts 1985 (125)	22	Single-center crossover	7 d	+

BCAA, branched-chain amino acids; +, some beneficial effect on encephalopathy with treatment; =, no differences between treatment and control.

Nonabsorbable Disaccharides

Lactulose (β-galactosidofructose) was first introduced with the aim of promoting the growth of *Lactobacillus bifidus*, which contains some urease activity, and, through urease, decreasing the production of ammonia in the colon. This is, however, not its mechanism of action, which is still complex and not fully understood. The bulk of evidence links the efficacy of lactulose to an interaction with the enteric flora and to a decrease in the generation of nitrogenous compounds in the intestine (126). Administered orally, lactulose is not broken down by intestinal disaccharidases and reaches the cecum, where it is metabolized by enteric bacteria to lactate and acetate (127), causing a drop in cecal pH. The decrease in pH appears to be necessary for lactulose to be active. Measurement of

stool pH can be used to monitor the dose, but is not practical. As a result of the changes induced in nitrogen metabolism in the colonic flora, lactulose increases fecal nitrogen excretion and decreases the amount of nitrogen that reaches portal blood (128). Subsequently, plasma levels of ammonia (the putative toxin) decrease (129). A similar mechanism of action is shared by other nonabsorbable disaccharides that are metabolized by the colonic flora, such as lactitol (β-galactosidosorbitol).

Lactulose is considered the "gold standard" in the treatment of HE, and drugs introduced for the management of HE are invariably compared with lactulose. However, the effectiveness of lactulose has never been validated by well-designed trials including a large number of patients (108). Few randomized studies have compared lactulose against placebo (Table 20.8). Nevertheless, the clinical experience with lactulose is large, and it is considered that clinical improvements should be expected in 70% of treated patients (130). Comparisons of lactitol to lactulose in randomized trials show a similar efficacy but better palatability for the former compound (131).

Table 20.8. Controlled Trials of Nonabsorbable Disaccharides and Neomycin for Hepatic Encephalopathy in Patients with Cirrhosis

Treatment	Control	Type of hepatic encephalopathy	Trial	*N*	Design	Duration	Effects on encephalopathy
Lactulose	Placebo (glucose)	Acute	Simmons et al. 1970 (132)	21	Parallel	10 d	+
Lactulose	Placebo (sorbitol)	Chronic	Elkington et al. 1969 (133)	7	Crossover	6 d	+
Lactulose	Placebo (saccarose)	Chronic	Germain et al. 1973 (134)	18	Parallel	To maximal improvement	+
Lactitol/lactose enemas	Cleansing enemas	Acute	Uribe et al. 1987 (135)	20[a]	Parallel	To maximal improvement	+
Lactitol enemas	Lactose enemas	Acute	Uribe et al. 1987 (135)	40	Parallel	4 d	=
Neomycin + starch enemas	Lactose enemas + placebo	Acute	Uribe et al. 1981 (136)	18	Parallel	5 d	=
Neomycin	Placebo	Acute	Strauss et al. 1992 (137)	39	Parallel	To maximal improvement	=
Neomycin + sorbitol	Lactulose	Acute	Atterbury et al. 1978 (138)	45	Parallel	To maximal improvement	=
Neomycin + lactulose	Placebo	Acute	Blanc et al. 1994 (139)	80	Parallel	5 d	=
Neomycin + sorbitol	Lactulose	Chronic	Conn et al. 1977 (140)	33	Crossover	10 d	=
Neomycin + magnesium sulfate	Lactulose	Acute + chronic	Orlandi et al. 1981 (141)	173	Parallel	To maximal improvement	=
Lactitol	Lactulose	Acute	Morgan 1987 (142)	28	Parallel	5 d	=
Lactitol	Lactulose	Acute	Heredia et al. 1987 (143)	40	Parallel	5 d	=
Lactitol	Lactulose	Chronic	Blanc et al. 1992 (131)	77	Meta-analysis	3–6 mo	=
Lactitol	Lactulose	Chronic	Camma et al. 1993 (144)	72	Meta-analysis	1–6 mo	=

[a]Analysis of the first 20 patients of the study.
+, some beneficial effect on encephalopathy with treatment; =, no differences between treatment and control.

Neomycin

Neomycin is considered an alternative drug to nonabsorbable disaccharides. It may be prescribed for patients who do not tolerate nonabsorbable disaccharides or when it is difficult to monitor their effects, for example, when a patient has diarrhea caused by an associated disorder or drug. Like lactulose, neomycin was introduced

to affect the intestinal flora that generates ammonia (138). However, the mechanism of action of neomycin may be through a nonbacterial effect (145). Neomycin has many effects on the intestinal mucosa and may even result in intestinal malabsorption. Because neomycin is an aminoglycoside, the major concern with its use is the potential for renal or auditory toxicity. Absorption of neomycin is poor (<4%), and the drug is considered potentially toxic only after long-term use. Toxicity may be minimized by tapering the dose after clinical response (i.e., neomycin 3 to 6 g/day during 2 to 3 days followed by 1 to 2 g/day thereafter) and avoiding prolonged use. The effect of long-term therapy is unclear. Periodic assessment of auditory and renal function, special nutritional care, and dose adjustment of additional drugs are recommended.

Clinical studies have demonstrated that neomycin exhibits efficacy similar to lactulose (Table 20.8). In acute episodes of HE, the efficacy of neomycin and lactulose is difficult to evaluate because the correct identification and treatment of the precipitating factor is the most important therapeutic measure. In a double-blind randomized study (137) comparing neomycin to placebo in patients with acute, but mild encephalopathy (approximately 70% stage I to II encephalopathy), neomycin did not significantly shorten the time to regression to a normal mental state. However, the duration of this period was variable, highlighting the difficulties in performing clinical trials in such patients because the course of the precipitant event cannot be fully controlled during randomization.

Flumazenil

The proposal that HE was related to an enhanced GABAergic tone was followed by the introduction of flumazenil, a highly selective antagonist of the central benzodiazepine receptor. Flumazenil is easy to administer and has few secondary effects. An arousal effect has been demonstrated in clinical trials (Table 20.9) and in experimental models (23). However, its clinical benefits are questionable because the drug causes only transient improvements of the mental state and is efficacious only for a subset of patients (146). When there is a response to flumazenil, it occurs within few minutes of administration of the bolus. However, in clinical studies no differences between placebo and flumazenil were seen 24 hours after the start of therapy (147). New antagonists, chemically related to flumazenil but with slightly different pharmacokinetic and pharmacodynamic properties, may be more effective for the management of encephalopathy.

Table 20.9. Controlled Trials of Flumazenil for Acute Hepatic Encephalopathy

	Flumazenil		Placebo		
Trial	***N***	**Responses (%)**	***N***	**Responses (%)**	**Effects on encephalopathy**
Cadranel et al. 1995 (146)	18	55	12	16	+
Pomier-Layrargues et al. 1994 (147)	13	46	15	0	+
Gyr et al. 1996 (148)	14	35	11	0	+
Barbaro et al. 1998 (27)	265	32	262	5	+

+, some beneficial effect on encephalopathy with treatment; =, no differences between treatment and control.

A source of controversy is whether the benefits of flumazenil reflect a potential antidote of the effects of exogenous benzodiazepines. In clinical trials the response to flumazenil was not related to detectable levels of benzodiazepines in plasma. Antagonists of the GABA receptor complex have resulted in amelioration of HE in animal models that were not given pharmaceutical benzodiazepines (23). Possible nonspecific effects of flumazenil must be evaluated in the management of other forms of metabolic encephalopathies.

Other Measures

Several additional treatments have been reported to be beneficial for HE. However, the use of these drugs is not widespread, probably because they do not present major advantages over nonabsorbable disaccharides. These drugs may be classified according to the main site of action (Table 20.10).

Decreasing the production of toxins

Several antibiotics have been used to treat patients with HE. They are aimed at reducing the intestinal flora, thereby decreasing the

source of intestinal toxins. It is intriguing that metronidazole, rifaximin, and vancomycin, antibiotics that affect bacterial populations different from those affected by neomycin, have been reported to improve encephalopathy (149,150,163). An important limitation of antibiotics is the risk for toxicity and the possible selection of multiresistant strains. For these reasons, antibiotics are not usually recommended for prolonged periods. Dose adjustments may be necessary for drugs that undergo hepatic elimination, such as metronidazole (250 mg twice a day).

Modification of intestinal flora with the aim of reducing ammonia production can also be achieved with agents that are not antibiotics, but experience with these agents is scant. Acarbose is a hypoglycemic agent

acting through the inhibition of glucose absorption that results in the promotion of intestinal saccharolytic bacterial flora at the expense of proteolytic flora. A randomized study found that acarbose significantly decreased ammonia blood levels and improved an intellectual score (154). The administration of probiotics (non–urease-producing *Lactobacillus* species) or fiber modifies the intestinal flora. Improvements in response to minimal HE (155) and chronic HE (156) have been observed with this approach, which may be related to a decrease in endotoxemia and secondary to an improvement in liver function.

Table 20.10. Drugs with Possible Beneficial Effects for Hepatic Encephalopathy

Drug	Mechanism of action	Trials	Patients (*N*)	Control	Effect
Metronidazole	Decreasing the production of toxins	Morgan et al. 1982 (149)	36	Neomycin	=
Vancomycin	Decreasing the production of toxins	Tarao et al. 1990 (150)	24	Lactulose	=
Rifaximin	Decreasing the production of toxins	DiPiazza et al. 1991 (151)	28	Neomycin	=
		Pedretti et al. 1991 (152)	30	Lactulose	=
		Bucci et al. 1993 (153)	58	Lactulose	+
Acarbose	Decreasing the production of ammonia	Gentile et al. 2005 (154)	107	Placebo	+
Synbiotic preparation[a]	Decreasing the production of toxins	Liu et al. 2004 (155)	55	Placebo	+
Enterococcus faecium SF68	Decreasing the production of toxins	Loguercio et al. 1995 (156)	40	Lactulose	+
Ornithine–aspartate	Fixation of ammonia	Kircheis et al. 1997 (157)	126	Placebo	+
Zinc + lactulose	Fixation of ammonia	Bresci et al. 1993 (158)	90	Lactulose	=
		Reding et al. 1984 (159)	22	Placebo + lactulose	+
		Riggio et al. 1991 (160)	15	Placebo + lactulose	=
Benzoate	Fixation of ammonia	Sushma et al. 1992 (161)	74	Lactulose	=
Bromocriptine	Activation of dopaminergic neurotransmission	Uribe et al. 1979 (162)	7	Placebo	=
		Morgan et al. 1980 (55)	6	Placebo	+

[a]4 non–urease producing bacteria plus fiber.
+, the effect of the drug on hepatic encephalopathy better than control; =, the effect of the drug on hepatic encephalopathy does not differ from control.

Fixation of ammonia

An increase in the capacity of a diseased liver to clear putative toxins is a desirable goal, but is difficult to attain. Activation of the urea cycle has been pursued as a measure to reduce blood ammonia levels. *Ornithine–aspartate* provides substrates both for ureagenesis and synthesis of glutamine, the two hepatic mechanisms that remove ammonia from the portal blood. The drug appears to prevent the increase in blood ammonia levels after a nitrogenous load and has been shown to be better than placebo in a study of episodes of HE in patients with cirrhosis (157). *Zinc*—a cofactor of urea cycle enzymes—is frequently deficient in cirrhosis, as a result of increased urinary excretion and malnutrition. Zinc supplementation (600 mg/day) has been proposed as a measure to reduce blood levels of ammonia and manage HE. The clinical results have been conflicting (159,160). Alternative pathways for nitrogen excretion, such as drugs used in children with urea cycle enzyme deficiencies (e.g., *benzoate* and *phenylbutyrate*), have been examined in cirrhosis. In the liver, benzoate is conjugated with glycine to form hippuric acid and phenylacetate (derived from phenylbutyrate) is conjugated with glutamine to form phenylacetylglutamine. Urinary excretion of these conjugates results in the removal of one and two nitrogen atoms per molecule of drug. Benzoate has been reported to be as efficacious as lactulose for the treatment of acute episodes of encephalopathy (161).

Drugs that act on the central nervous system

Drugs that enhance dopaminergic neurotransmission were introduced to restore the proposed displacement of central neurotransmitters caused by the putative false neurotransmitters. Although the hypothesis was contested in subsequent experimental observations, recent evidence supports the existence of dopaminergic dysfunction that may arise from the accumulation of manganese in the basal ganglia (164). When targeted to improve consciousness, dopaminergic drugs (e.g., levodopa, bromocriptine) did not yield satisfactory results (162). However, they may have a role in the treatment of extrapyramidal manifestations in patients with chronic encephalopathy. In these subjects, improvements of extrapyramidal signs have been reported when bromocriptine is added to conventional

therapy (55). The constipation caused by bromocriptine may be counteracted by an increased dose of nonabsorbable disaccharides.

Management of the Acute Episode of Hepatic Encephalopathy

Diagnosis

The diagnosis of HE is clinical and relies on the development of compatible neurologic manifestations in a patient who has severe liver failure and/or portosystemic shunting. The development of any neurologic abnormality in patients with cirrhosis should raise the possibility of HE. However, there is no diagnostic test that confirms the clinical suspicion. The diagnosis is supported by the presence of a time-related precipitating factor and by a history of similar episodes. However, the neurologic manifestations may vary from the first to subsequent episodes (165).

The neurologic manifestations of HE are not specific and can be present in many other metabolic or structural types of encephalopathy. Patients with alcoholic cirrhosis may have alcohol-induced complications, such as Wernicke-Korsakoff encephalopathy, seizures, alcoholic intoxication, or deprivation. For these reasons, the first step is to exclude alternative diagnoses. Usually, the clinical history, the physical examination, and routine blood tests are enough to exclude other neurologic diseases. Additional tests are indicated according to the clinical situation (Table 20.11). A common pitfall is not to diagnose thiamine deficiency, as emphasized by results of a neuropathologic study of patients with cirrhosis who died in coma (166). The determination of the activity of pyruvate transketolase in blood and the routine administration of thiamine may help in these circumstances. A CT scan is recommended to exclude structural abnormalities in patients with focal neurologic signs, severe encephalopathy, or lack of precipitating factors or in those who do not recover after adequate treatment is initiated. EEG is not helpful for establishing the diagnosis of HE because slowing of the normal rhythm is common in other forms of encephalopathy. Occasionally, the results of the EEG may suggest other diseases, such as status epilepticus or the development of herpetic encephalitis. Examination of the CSF may be helpful in select cases to rule out infectious meningoencephalitis, but lumbar puncture is complicated by concomitant coagulopathy. It is important to emphasize that the clinical course may fluctuate and that frequent observation of the patient is necessary. Assessment of the stage of HE is helpful to follow the evolution.

Table 20.11. Differential Diagnosis of Hepatic Encephalopathy

Alternative diagnosis	Clinical clues
METABOLIC ENCEPHALOPATHIES	
Hypoxia or hypercapnia	Cyanosis, respiratory signs, blood gas
Hypoglycemia	Hepatocarcinoma, diabetic therapy, clinical chemistry
Hyponatremia or hypernatremia	Diuretic therapy, body weight changes, blood chemistry
Azotemia	Diuretic therapy, vomiting, blood chemistry, urinalysis
Diabetic coma	Diabetes, blood chemistry
INTRACRANIAL STRUCTURAL DISORDERS	
Stroke	Focal neurologic signs, neurologic imaging (e.g., CT scan, MRI)
Subarachnoid hemorrhage	Sudden headache, arterial hypertension, lumbar puncture, neurologic imaging (e.g., CT scan, MRI)
Intracranial tumors	Focal neurologic signs, neurologic imaging (e.g., CT scan, MRI)

Subdural hematoma	Alcoholism, cranial trauma, focal neurologic sings, neurologic imaging (CT scan, MRI)
DRUG OR TOXINS	
Alcohol	Drug abuse, urine and blood toxin screen
Hypnotics	Drug abuse, urine and blood toxin screen
MISCELLANEOUS	
Meningitis, encephalitis, cerebral abscess	Fever, meningeal signs, lumbar puncture, neurologic imaging (e.g., CT scan, MRI)
Seizures	Prior history, bitten tongue, urinary incontinence, EEG
Wernicke's encephalopathy	Alcoholism, pyruvate transketolase activity, response to thiamine, compatible MRI signs
Alcohol withdrawal	Prior history, visual hallucinations, history

CT, computed tomography; MRI, magnetic resonance imaging; EEG, electroencephalography.

The diagnosis of HE requires the appreciation that the patient has underlying hepatic dysfunction. Usually, this conclusion can be reached with a clinical history, physical examination, and laboratory testing. However, some patients with normal or nearly normal liver test results may have fully established cirrhosis and/or large portosystemic shunts. The finding of spider nevi or mild thrombocytopenia may indicate the presence of underlying cirrhosis. CT scan or ultrasonography shows prominent venous collateral vessels or an irregular liver. Arterial ammonia levels add nothing in terms of diagnosis of typical cases, but,

occasionally, may be the clue for diagnosing a congenital portosystemic shunt, an acquired urea cycle enzyme defect presenting in adults or significant liver disease. Care to avoid false-positive values requires proper handling of samples and adequate performance of assays.

Supportive Measures

The supportive measures include the general management of a patient with change in mental status. Intravenous catheters are usually necessary to provide fluid and electrolytes. Hydration is important; patients should receive adequate fluid replacement, even if they present with ascites or edema. Replacement of intravenous fluid with albumin infusion appears to be superior to the use of other volume expanders for patients with diuretic-induced encephalopathy (19). In general, diuretics should be avoided, unless pulmonary edema is present. Urinary and nasogastric tubes may be necessary. Special care should be taken to avoid line sepsis. In deep coma, the prevention of aspiration pneumonia may require tracheal intubation and ventilatory support. In immobile patients, pressure sore prevention is necessary.

It is important to provide an adequate nutritional support. The current recommendation is to provide 25 to 35 kcal/kg per day or 0.5 to 1.2 g/kg per day of proteins or amino acids, respectively (110). In most cases during the initial period of decreased arousal (2 to 3 days), the patient is initially treated exclusively with glucose supplements as intravenous fluids. Usually, after this period the patient recovers the ability to tolerate an oral diet. The administration of diet with a normal content of protein has been shown to be safe (111). Therefore, patients should receive standard diets with 60 to 80 g/day of proteins. Patients with prolonged coma (>2 to 3 days) should receive nitrogen supplements as solutions of amino acids—up to 70 g/day—preferably through the enteral route (167).

Table 20.12. Management of Precipitating Factors

Precipitating factor	Diagnostic approach	Management
Gastrointestinal bleeding	Examination of rectal and gastric content Endoscopy	Specific treatment according to site of hemorrhage Bowel cleansing
Constipation	Clinical history	Bowel cleansing
Large protein meal	Clinical history	Bowel cleansing
Psychoactive drugs	Clinical history Drug screen	Antidotes (i.e., flumazenil, naltrexone)
Renal failure	Renal function tests Ultrasonography	Withhold diuretics and nephrotoxic drugs Specific treatment of the cause

Electrolyte imbalance	Blood electrolytes	Withhold diuretics Correct electrolyte disturbances
Infection	Cultures of blood and body fluids Tap of ascites and pleural effusion	If suspicious, broad-spectrum antibiotics (avoid aminoglycosides) Adjust antibiotics to microbiologic studies
Superimposed acute hepatic injury	Clinical history Liver chemistries, ultrasonography Liver biopsy	Specific measures may be helpful according to cause

The table is conceived as a guide for the recognition and management of the most common precipitating factors; it does not include an exhaustive description of the diagnostic and therapeutic procedures.

Elimination of Precipitating Factors

The clinical course of HE can be interrupted in the most patients through control of precipitating factors (Table 20.5). Effective methods exist to control most of these factors. The key is identifying their presence. Multiple precipitating factors may be present and some may be occult and difficult to demonstrate. Systematic exclusion of all the precipitating factors is recommended (Table 20.12) but can be difficult because of the situation of the patient. A practical approach is to initiate several therapeutic measures against putative precipitating factors while additional information and the results of the complementary tests become available.

A common pitfall is to not exclude an ongoing infection. Cultures of blood and body fluids and cell count of ascitic fluid are recommended even in the absence of clear signs of infection. It is wise to assume that a patient with cirrhosis and a major change in mental status is infected until proved otherwise. If a patient does not have a clear precipitating factor, broad-spectrum antibiotics may be initiated until the results of the cultures become available.

Constipation and a large oral protein load are common precipitating factors that may be difficult to recognize. HE may be associated with a slow intestinal transit and, through this mechanism, amplify the generation of encephalopathic substances from the intestine (168). Colonic cleansing reduces the luminal content of ammonia, decreases bacterial counts, lowers blood ammonia, and prevents encephalopathy after a gastrointestinal hemorrhage (169). It is common to include bowel cleansing as part of the initial therapy. Various laxatives can be used, but nonabsorbable disaccharides are preferred because they result in additional effects that enhance the elimination or reduce the formation of nitrogenous compounds. Precipitation of HE by major gastrointestinal hemorrhage is generally recognized easily. However, it is sometimes not evident until intestinal cleansing is commenced. It is important to remember in such cases that treatment includes not only arrest of hemorrhage but also the administration of broad-spectrum antibiotics to prevent superimposed infection.

Administration of Drugs

Lactulose has become the standard drug for the treatment of acute episodes of encephalopathy. We have reviewed the uncertainty of its benefits in this setting (108). Initially, patients should receive a large dose of lactulose or another nonabsorbable disaccharide (142) orally (50 mL of lactulose syrup every 1 to 2 hours) or as an enema (300 mL lactulose in 1 to 3 L of water). After catharsis begins, the oral dose (15 to 30 mL lactulose four times a day) or dose of the enema (i.e., every 8 to 12 hours) should be adjusted. Patients in coma or with small bowel ileus can receive lactulose by enema (135). The administration of lactulose in enema shortens the time to achieve catharsis. However, the major limitation of enema is the difficulty in administering it correctly, especially in semiconscious patients. The patient should be placed in Trendelenburg left lateral decubitus position, then right decubitus, and finally a position in which the upper part of the body is elevated to maximize the exposure of the entire colon. The dose of nonabsorbable disaccharides—orally or through a nasogastric tube—is titrated to produce two to three soft bowel movements daily. If diarrhea develops, the drug should be stopped and reinstituted later at a lower dose. Protracted diarrhea can result in hypertonic dehydration with hypernatremia (170).

Although the use of flumazenil in HE is not well standardized, a therapeutic trial of flumazenil can be attempted. This is justified by the prescription of pharmaceutical benzodiazepines to hospitalized patients with cirrhosis and the impossibility to identify a priori those patients who may respond. Flumazenil seems not to dramatically modify the course of HE because in clinical trials there were no differences with placebo 24 hours after its administration. However, it may be useful to improve consciousness and avoid orotracheal intubation in patients in deep coma. Flumazenil is available as an intravenous preparation that is administered as a bolus (0.4 to 2.0 mg). If a favorable response occurs, additional doses can be given, but the effects of multiple doses of flumazenil have not been evaluated. Dose adjustments may be necessary because elimination half-life (normal individuals approximately 45 minute) may be doubled in patients with cirrhosis. Overdose of flumazenil may have proconvulsant effects.

Management of the Patient with Chronic Hepatic Encephalopathy

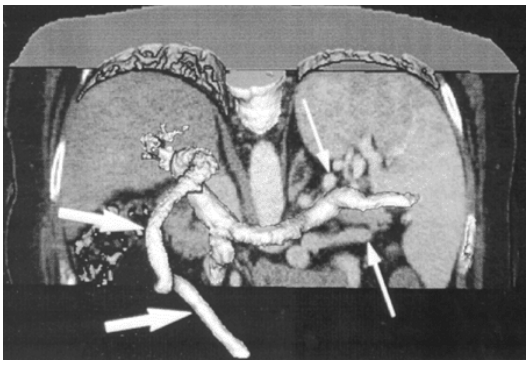
Diagnostic Problems

One of the most difficult diagnostic problems is to differentiate chronic HE from other chronic degenerative diseases of the CNS in patients with chronic liver failure. A fluctuating course with relapsing episodes of encephalopathy is characteristic of chronic relapsing HE. However, patients with chronic persistent HE may show neither a fluctuating course nor superimposed relapsing episodes. The differential diagnosis with neurodegenerative diseases is difficult because most of them are diagnosed on clinical grounds. Although a therapeutic trial may be attempted, the lack of response is not necessarily an argument against HE. MRI of the brain may be helpful in select cases (171). The absence of hyperintensity of the basal ganglia on T_1-weighted imaging and the characteristic pattern of decreased *myo*inositol and increased glutamine/glutamate concentrations in spectroscopy are arguments against the diagnosis of HE.

In the care of patients with chronic relapsing HE, it is important to differentiate those with precipitated and those with spontaneous events. Several covert precipitating factors, such as over-the-counter medications or diuretic overdose, may not have been recognized. In patients with apparent spontaneous encephalopathy and those with chronic persistent HE it is reasonable to presume that they have associated large portosystemic shunts before assuming that HE is the irreversible manifestation of terminal liver disease. In this setting, ultrasonography and abdominal CT scan may be helpful in revealing a large portosystemic shunt that may be amenable to radiologic occlusion (Fig. 20.10).

Among the additional factors that underlie the relapsing episodes of HE, it is important to consider the nutritional state, especially in the care of patients with alcoholism. Trials with zinc administration have not shown impressive results on the course of HE. However, it may be worthwhile to assess plasma

zinc levels and administer zinc supplements to patients with low levels. Colonization of the stomach with *Helicobacter pylori* is frequent in patients with cirrhosis. Because it is a urease-containing organism, it has emerged as a possible source of hyperammonemia and HE. Treatment of *H. pylori* infection may be attempted in chronic cases. However, results of several studies suggest that eradication of *H. pylori* is not associated with improvements in HE or a decrease in plasma levels of ammonia (172).



▪ **Figure 20.10** Three-dimensional reconstruction of the portosplenic venous circulation and a patent umbilical vein combined with a coronal reconstruction of a helical CT scan. The figure shows a small liver, an enlarged spleen and a patent portal vein that is mildly dilated. Large portosystemic shunts are seen at the level of the umbilical vein (*thick arrows*) and perisplenic collateral veins (*thin arrows*). (From Cordoba J, Hinojosa C, Sanpedro F, et al. Usefulness of magnetic resonance spectroscopy for diagnosis of hepatic encephalopathy in a patient with relapsing confusional syndrome. *Dig Dis Sci* 2001;46:2451–2455, with permission.)

Design of a Therapeutic Program

Patients with chronic HE usually exhibit severe malnutrition. Therefore, the routine prescription of protein-restricted diets may worsen the nutritional status (173). Patients are better off with multiple small feedings (five to six per day). Evening snacks are recommended, and the importance of breakfast should be stressed. The amount of protein in the diet should be individually adapted. Progressive increments on the total amount of proteins should be tried. Tolerance to protein may be improved by feeding dairy products and vegetable-based diets. Oral branched-chain amino acids are reserved for the protein-intolerant patient. In one study including 64 patients with cirrhosis with mild forms of chronic encephalopathy (124), treatment with oral branched-chain amino acids was associated with a decreased number of acute exacerbations and nutritional improvement. Treatment was maintained for 3 months and complemented with lactulose and a limited protein intake (45 to 65 g/day). However, other studies—with a smaller sample size and a shorter duration—have not yielded consistent results (114).

Nonabsorbable disaccharides should be prescribed and the patient should be instructed to adjust the dose to obtain two to three bowel movements per day. The oral dose should be frequently augmented during chronic treatment because of individual variability and changes in the bacterial metabolism of disaccharides (127). Care is needed to avoid excessive diarrhea and dehydration-precipitated HE. Some patients complain of an excessively sweet taste, flatulence, or abdominal cramping and find treatment with nonabsorbable disaccharides difficult to follow. Neomycin or other antibiotics may be an alternative for these patients. Tests to monitor toxicity should be periodically performed and periods of more than 6 months under the same antibiotic should be discouraged.

Management of problems associated with chronic liver dysfunction are of special relevance for patients with chronic HE. Ascites is better treated with paracentesis than diuretics. Nevertheless, low-dose diuretics may be prescribed. Plotting the patient's increase in body weight can be helpful. It is important for the patient to understand that some mild degree of water retention is not harmful and it is better to have some degree of edema than to suffer an episode of HE. Among different alternatives to prevent variceal bleeding, pharmacologic or endoscopic treatments are less likely to cause HE as compared to decompressive options (e.g., TIPS, surgical shunting).

The development of HE carries important prognostic implications (174). All patients who have suffered an episode of HE should be considered liver transplantation candidates. In patients with chronic encephalopathy, the decision to proceed to liver transplantation can be difficult. Severe chronic

neuropsychological abnormalities are usually considered a contraindication for liver transplantation. However, improvement of such manifestations have been reported after transplantation (175). Neuropsychological abnormalities are well documented after liver transplantation. The controversy is whether they correspond to sequelae of HE or are the neurologic consequences of perioperative complications (176). The surgical exclusion of the colon was proposed for chronic severe HE (177). However, this measure has been abandoned because of a high surgical mortality.

The Patient with Portosystemic Shunts

A common subset of patients with chronic HE are those with *postshunt encephalopathy*, secondary to a previous surgically created portosystemic shunt or TIPS. Persistent and intractable encephalopathy may be treated by occluding the shunt (80,178). In patients who have undergone a TIPS, a prudent waiting time is warranted. Most episodes of encephalopathy are concentrated during the first 2 months after the procedure and usually respond to treatment with lactulose; subsequent narrowing of the stent—an untoward hemodynamic effect—may afford protection from encephalopathy (179). The prophylactic administration of drugs to prevent the development of encephalopathy after the insertion of TIPS has not been shown to be effective (180). If encephalopathy becomes problematic, the stent diameter can be reduced (80,181). The risks of reintervention and rebleeding after shunt reversal should be weighed against the severity of the neurologic symptoms. Newer, coated stents with a decreased incidence of narrowing may result in more problems with HE in the future.

Occlusion of portosystemic shunts should be also considered in the rare patient with cirrhosis and large spontaneous shunts or in

the exceptional patient with congenital shunts (182). In patients with cirrhosis it is preferred to occlude large shunts with angioradiologic techniques because they have lower operative risk. Several reports suggest that it is a safe and effective treatment in select patients. Treatment of congenital shunts depends on the characteristics of the portal vein and may be performed with surgical or angioradiologic approaches. Closure of the shunts associated with marked hypoplasia of the portal vein can cause portal hypertension and gastrointestinal bleeding. Slow closure of the shunt may be more adequate (183).

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Chapter 21
Fulminant Hepatic Failure

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Key Concepts

- Fulminant hepatic failure (FHF) is defined by the presence of hepatic encephalopathy occurring as the consequence of severe liver damage in patients without previous, clinically overt liver disease.
- Components of the clinical syndrome include cerebral edema, hemodynamic instability, renal failure, coagulopathy, metabolic disturbances, and susceptibility to bacterial and fungal infection.
- The nature of the causative factor or initiating event is an important factor influencing both the rate of progression of the clinical syndrome and its prognosis. Establishing the cause is also important because for certain etiologies specific therapies can be effective.
- Relative prevalences vary according to geographic location, with hepatotoxicity due to acetaminophen and idiosyncratic drug hepatotoxicity being the most common cause in many western countries and single or dual infection with the hepatitis viruses predominating in the East. The cause is not established in 10% to 20% of patients, in whom non–A to E viral hepatitis or an undetected hepatotoxin is presumed to be responsible.
- Supportive management, based on an understanding of underlying pathophysiology, is aimed at maintaining hemodynamic, cerebral, and renal function, reversing metabolic derangements, preventing or treating complicating infection, minimizing stress ulceration of gastric mucosa, and, where appropriate, treating coagulopathy.
- In its most severe form, FHF carries a high mortality rate unless urgent liver transplantation is performed. Lack of fulfillment of current selection criteria cannot be taken to reliably predict spontaneous survival, making it difficult to identify those patients who can confidently be managed by medical means alone.
- The efficacy of temporary liver support based on the use of artificial or bioartificial devices, either as a bridge to emergency transplantation or to allow time for the regeneration of the native liver, upon which spontaneous survival ultimately depends, remains to be proved in controlled clinical trials.

The original description of fulminant hepatic failure (FHF) in 1970 was based on the occurrence of hepatic encephalopathy as a consequence of severe liver injury developing within 8 weeks of the onset of symptoms in patients without preexisting liver disease (1). More recent terminologies taking into account the interval between the onset of jaundice, rather than often nonspecific symptoms at onset, and the development of encephalopathy have alternatively been proposed (Table 21.1) in recognition of the fact that the jaundice-to-encephalopathy time is an important prognostic index (2,3,4). In contrast to the original description,

these other classifications allow for the inclusion of cases with previously asymptomatic

chronic liver conditions, such as fulminant presentations of Wilson disease and underlying chronic hepatitis B. In this chapter, FHF is applied as a generic term encompassing this range of definitions.

Table 21.1. Definitions of Fulminant Hepatic Failure Based on the Time Interval Between the Onset of Jaundice and the Development of Hepatic Encephalopathy, an Important Prognostic Guide

Authors	Classification	Jaundice-to-encephalopathy time
Bernuau et al. Paris (2)	"Fulminant liver failure"	Within 2 wk
	"Subfulminant liver failure"	Between 2 and 12 wk
O'Grady et al. London (3)	"Hyperacute liver failure"	Within 1 wk
	"Acute liver failure"	Between 1 and 4 wk
	"Subacute liver failure"	Between 5 and 12 wk

FHF is a potentially devastating syndrome that may include as components cerebral edema, hemodynamic instability, renal failure, coagulopathy, profound metabolic disturbances, and a particular susceptibility to bacterial and fungal infection. The severity of the clinical syndrome in individual patients depends on the degree of impairment of metabolic and endotoxin-scavenging activity consequent to the loss of functioning liver tissue, together with the systemic effects of various cytokines released by activated nonparenchymal cells, and the extent to which these abnormalities are reversed by liver regeneration, upon which the prospect of spontaneous survival ultimately depends. The nature of the initiating event is an important factor influencing both the histologic pattern of liver injury and the rate of progression of the clinical syndrome. Although advances in supportive medical care have improved survival, FHF, in its most severe form, continues to carry a high mortality rate unless emergency orthotopic liver transplantation (OLT) is performed. Nonetheless, the rapidity with which the clinical syndrome often progresses, along with a worldwide shortage of cadaveric donor organs, is such that many patients die or develop contraindications to the transplantation before a donor liver becomes available, even with priority listing, thereby limiting the number that can be treated in this way. The increasing use of living-donor liver transplantation in recent years is another option that needs to be considered for adults and children with FHF. The risk of serious side effects from long-term pharmacologic immunosuppression means that conventional OLT cannot be considered a panacea in this setting, and there is considerable ongoing interest in the provision of temporary liver support by auxiliary partial liver transplantation, extracorporeal artificial or bioartificial devices, or hepatocyte transplantation, with the ultimate aim of allowing time for or even actively promoting native liver regeneration, thereby decreasing the need for OLT.

This chapter focuses on the range of etiologies of FHF and their influence on both clinical manifestations and prognosis, the spectrum of liver damage along with factors influencing liver regeneration, and the pathophysiology of the resultant clinical syndrome. Management issues are discussed, including specific and supportive medical measures of proved value, indications for OLT, and the current status of evolving strategies for temporary liver support.

Etiology and Related Clinical Implications

The prevalences of the various etiologies of FHF vary according to geographic location. The natural history of FHF is influenced by its cause (5,6,7). In addition to carrying prognostic significance, establishing the cause whenever possible is important so that specific therapies can be instituted, at least in a proportion of cases (Table 21.2). Experience in the United Kingdom, other European countries, and the United States has shown that the rate of progression of the clinical syndrome varies according to the etiology and, somewhat paradoxically, that spontaneous survival with medical management alone is inversely related to the rapidity of onset of encephalopathy (5,6,7). In a nontransplantation

series, survival was 36% when encephalopathy was hyperacute in onset, occurring within 1 week of the development of jaundice, but no more than 14% with longer jaundice-to-encephalopathy times. FHF due to acetaminophen hepatotoxicity is nearly always hyperacute in onset, as are most cases related to infection with hepatitis A virus (HAV) and hepatitis B virus (HBV) and a lower proportion of patients with the other etiologies discussed in the subsequent text (3). Owing to the rapidity of progression of encephalopathy in the hyperacute category, patients may become comatose before clinical jaundice is even apparent. Within the acetaminophen hepatotoxicity group, survival with medical management is inversely correlated with the admission grade of encephalopathy, at least in nonacidotic patients, highlighting the potential benefit of early referral (4,6,8).

Table 21.2. Etiology-Specific Therapies to be Considered in Patients with Fulminant Hepatic Failure

Etiology	Therapy
Acetaminophen	*N*-acetylcysteine
Hepatitis B virus	Lamivudine, famciclovir
Herpes simplex virus	Acyclovir
Cytomegalovirus	Ganciclovir
Autoimmune hepatitis	Trial of corticosteroids, cyclosporine
Pregnancy associated	Urgent delivery
Budd-Chiari syndrome	Decompressive vascular shunt
	Thrombolysis
	Hepatic venous angioplasty
	Stenting of inferior vena cava
	Treatment of any underlying procoagulant state
Veno-occlusive disease	Decompressive vascular shunt
	Thrombolysis

Cardiac failure	Inotropes
Septic shock	Antibiotics, vasopressor agents
Wilson disease	D-penicillamine
Amanita phalloides	Penicillin, silibinin
Lymphoma	Chemotherapy

Acetaminophen

Hepatotoxicity due to acetaminophen accounted for 60% to 70% of cases in a recent series from the United Kingdom (9,10). Acetaminophen has also been reported to be the most common cause of FHF in recent series from Denmark, the United States, and many other western countries (6,11,12), reflecting the fact that acetaminophen remains the most commonly used substance causing self-poisoning in these countries (13,14,15,16). There is no evidence that increasing general awareness of the serious toxicity of acetaminophen leads to any reduced frequency of overdose (17). Indeed, the opposite may pertain. In the United Kingdom, rates of acetaminophen overdose with deliberate intent have increased in both men and women in comparison to the late 1980s, with rates in men showing a relatively larger increase and now approaching those in women (13,14,15,16,18,19). Recent legislative change in packaging and the introduction of measures designed to limit the quantity of acetaminophen that can be purchased without a prescription have had some impact in reducing the number of overdoses and also the number of patients with severe liver damage (20,21). An estimated 100,000 cases of intentional acetaminophen overdose are reported annually in the United States (22). Increasing numbers of overdose cases are also being recorded in Australia and many other parts of the world (23,24,25,26).

Most instances of acetaminophen-induced hepatotoxicity in the United Kingdom and Australia are the consequence of an overdose of the drug taken at a single time point with suicidal or parasuicidal intent (25,26,27,28,29,30,31). Cases of severe hepatotoxicity after ingestion of recommended (or near-recommended) doses of acetaminophen, mostly over several days to weeks, have also been reported, including those in patients with long-term exposure to alcohol or enzyme-inducing drugs, such as antituberculous chemotherapy (e.g., rifampicin and isoniazid) and anticonvulsants (e.g., phenytoin, carbamazepine, and phenobarbital) (12,32,33,34). Considerable emphasis has been placed on this in the United States, with the combination of alcohol and acetaminophen accounting for up to two thirds of all cases of FHF admitted to one center in a recent report (12).

A true effect of long-term alcohol use in predisposing to acetaminophen-related hepatotoxicity at low doses or in exacerbating liver injury in the overdose situation would be of great clinical significance, given the increased incidence of depression among patients with long-term, heavy alcohol intake (35) and the well-documented association between alcohol use and suicidal behavior. Up to 62% of patients hospitalized after a nonfatal suicide or parasuicide attempt in the United Kingdom, including 40% to 75% of men and 12% to 50% of women, have consumed alcohol at the time of the event or in the 6 hours before it (36,37,38,39). An association with recent alcohol use is also apparent in substantial proportions of patients presenting to Australian centers after deliberate acetaminophen overdose (25,26). Alcohol and acetaminophen are also frequently used together in the general community in the absence of suicidal or parasuicidal intent. Ten percent of long-term alcoholics admitted to a detoxification program in the United States were found to have recently consumed acetaminophen for pain relief (40).

Our experience in the United Kingdom, and that of others, is that most instances of FHF related to acetaminophen in alcoholics, as in nonalcoholic patients, are the direct

consequence of having ingested a large overdose of the drug at a single time (25,26,27,28,29,30,31). Most cases of severe hepatotoxicity occurring in association with therapeutic acetaminophen use, rather than suicidal or parasuicidal intent, are also caused by ingestion of large doses, generally over several days (41). A recent prospective analysis of patients presenting to centers in the United States with acetaminophen-related FHF found that the mean dose ingested by those taking acetaminophen for therapeutic purposes (30 g) was significantly higher than that taken by those with suicidal intent (15 g) (42). Large retrospective series to date have provided little evidence that long-term alcohol exposure exacerbates acetaminophen-related hepatotoxicity or worsens outcome when doses markedly in excess of the recommended daily maximum for acetaminophen have been ingested. In the United States, Rumack et al. (43) found no difference in the severity of hepatotoxicity in patients with and without chronic alcohol abuse among a national cohort of 662 consecutive patients presenting with "at risk" blood acetaminophen levels. Neither was a history of long-term alcohol consumption in excess of 80 g daily

associated with a significantly worse prognosis in a series of 247 patients managed at King's College Hospital, London, in 1982 and 1983, most of whom had allegedly ingested 25 to 50 g at a single time (31). A subsequent study of 79 patients presenting to the same unit in 1987 and 1988 with hepatotoxicity related to similarly large doses of acetaminophen (30) examined markers of severity of liver damage and outcome in relation to alcohol intake above or within the guidelines recommended by the Royal College of Physicians, namely 21 units per week for men and 14 units per week for women. Rates of development of cerebral edema and requirement for dialysis or ventilation were not significantly more prevalent in the high–alcohol intake group. The finding of an increased mortality rate in those with an alcohol intake above that recommended by the Royal College of Physicians than in those with more modest alcohol intake (67% vs. 34%, respectively) was not confirmed when these patients were included in a larger series of 553 patients with hepatotoxicity related to acetaminophen overdose who presented between 1987 and 1993 (27). Despite the large sample size, no association could be detected between long-term alcohol consumption and severity of hepatotoxicity, as reflected by rates of requirement for inotropic support or renal dialysis, development of grade III or IV encephalopathy, or fulfillment of transplantation criteria. Similarly, Denison et al. (41) failed to identify an association between long-term alcohol use and severity of liver injury after acetaminophen overdose in a series of 38 consecutive patients in Sweden. On the other hand, Smilkstein and Rumack (44) more recently reported higher serum levels of liver transaminases after paracetamol overdose in patients with a history of long-term alcohol intake than in those without.

The possibility that FHF may occur in long-term alcoholics after ingestion of acetaminophen at recommended or near-recommended daily doses, taken for therapeutic reasons, has been raised mainly in case reports from the United States (12,32,33,34). Zimmerman and Maddrey (45) retrospectively identified from a United States registry a cohort of 27 patients with acetaminophen-related hepatotoxicity after the ingestion of 4 g or less of paracetamol daily, mostly for periods in excess of 1 day and up to 1 week. All were regular users of alcohol, mostly in excess of 60 g daily. Schiodt et al. (46) retrospectively identified 21 patients who had accidentally poisoned themselves while taking acetaminophen for analgesia, generally using recommended or near-recommended doses repeatedly over days to weeks, among a total of 71 patients hospitalized for acetaminophen-related toxicity at an urban county hospital in the United States over a 3-year period to 1995. Patients in the "accidental" category had significantly higher rates of severe liver disease and death than their suicidal counterparts, despite consuming substantially lower amounts of acetaminophen, which the authors postulated was due to a greater proportion of long-term alcohol abusers in the former group (63% vs. 25%, respectively). Delays in recognition of the etiologic connection between acetaminophen use and hepatotoxicity contribute to a poor prognosis.

Cases of "accidental" or "therapeutic dosage" acet aminophen hepatotoxicity have also been reported in the absence of long-term alcohol use, mostly in the context of prior starvation or malnutrition (27,47,48,49). Indeed, in a study of 10 patients, identified over a 5.5-year period at hospitals of the University of Pittsburgh Medical Center, who had developed hepatotoxicity with paracetamol doses less than 10 g daily, Whitcomb and Block (49) found that liver injury correlated more closely with recent fasting than with long-term alcohol use. Most of these patients had ingested acetaminophen for more than 1 week before the onset of

symptomatic hepatotoxicity.

The development of FHF due to acetaminophen may be prevented if the antidote, *N*-acetylcysteine, is given within 15 hours of exposure. Furthermore, the later use of this agent, after signs of liver necrosis have developed, has been shown in one controlled trial to ameliorate associated multiorgan failure and improve survival (50).

Hepatitis Viruses

Any virus that can cause acute hepatitis may potentially give rise to FHF. Such viruses can be broadly categorized as those that primarily affect the liver, such as the hepatitis viruses A to E, and those in which liver involvement may occur as part of disseminated infection, as with Epstein-Barr virus, Cytomegalovirus (CMV), varicella-zoster virus, enteroviruses, parvovirus B19, adenovirus, and herpes simplex virus (HSV). The latter occur mainly, although not exclusively, in the immunosuppressed and in children (10,51). FHF due to HSV may respond to high-dose acyclovir (52), while treatment with ganciclovir is instituted in cases of CMV infection. FHF has also been reported in cases of infection with Toga virus–like particles, papilloma virus, paramyxoviruses, and hemorrhagic fever viruses (10,53). Although decreasing as a cause of FHF in the West, viral hepatitis remains the major etiologic factor in a large part of the world, including Asia, the western Pacific region, the Middle East, Africa, South America, and some European countries.

In general, the frequency with which the different hepatitis viruses cause FHF in a particular geographic location is a reflection of their underlying prevalence. Accounting for less than 10% of cases of FHF in most series, the risk of FHF due to HAV increases markedly in those older than 40 years and in the setting of preexisting chronic liver disease (54). HAV superinfection in

patients with chronic hepatitis C virus (HCV) infection resulting in FHF in one series from Italy was attributed to the HAV in view of a reduced rate of HCV replication observed during acute HAV infection (55). The implication of this finding is that those with chronic HCV infection should be vaccinated against HAV, although its cost-effectiveness is yet to be established (56).

HBV infection is highly endemic in the southeast Asian and western Pacific regions, along with parts of the Mediterranean Littoral, the Middle East, and sub-Saharan Africa (57) and, accordingly, is the major cause of FHF in such areas. Reactivation of HBV, such as after withdrawal of immunosuppressive or cytotoxic chemotherapy for various hematologic and other malignancies (58), is a well-recognized cause of FHF. Reactivation occurring in the context of chronic HBV carriage, unrelated to immunosuppression withdrawal, is more common than de novo HBV infection as a cause of FHF in Taiwan and other countries of the Far East. Antiviral agents, such as lamivudine, is usually given in patients with detectable circulating HBV deoxyribonucleic acid (DNA) levels, although viral replication is characteristically low or absent at the time of clinical presentation with FHF and reports are conflicting as to their value.

In fulminant HBV infection, there appears to be an exaggerated host immune response so that one half to two thirds of cases have lost hepatitis B surface antigen (HBsAg) within a few days of clinical presentation. The diagnosis of acute HBV infection in such cases rests on the presence in serum of immunoglobulin M antibody to hepatitis B core antigen (anti-HBc IgM). However, a recent Japanese study of patients with FHF found that anti-HBc IgM was not measurable in serum in approximately 50% of cases in whom HBV was detected by a sensitive polymerase chain reaction technique (59). Evidence of HBV infection in seronegative individuals with FHF, the so-called occult HBV infection, was first reported in San Francisco, where HBV DNA was detected in serum or liver tissue of 6 out of 17 (35%) such patients (60). HBV DNA was found in the liver, but not serum, in 50% of those for whom both serum and the liver were available for analysis. HBV viral sequences may even be evident in liver tissue when immunohistochemical staining for HBsAg and hepatitis B core antigen (HBcAg) is negative (61). Nonetheless, evidence of "occult" HBV infection was not found in any patient with FHF of otherwise "indeterminant" etiology in a more recent multicenter series of 22 such patients from the United States (62).

An association between FHF and the precore mutant HBV strain, which has a point mutation

at nucleotide 1896 in the precore region that results in a translational stop codon, preventing the secretion of hepatitis e antigen (HBeAg), has been described (63,64). This mutant is highly prevalent in Asia, Africa, and the Middle East (65), especially in relation to HBV genotype D (66,67). Interestingly, recent data from Taiwan suggest that acute superinfection of chronic HBV carriers with HCV suppresses the development of the precore stop mutant, in marked contrast to the progressive increase that occurs during the natural history of chronic HBV infection alone (68). Cases of FHF due to mutant HBV appear to be caused by transmission of the mutant virus rather than by a subsequent mutation of an infecting wild-type strain. The precore mutation has been found in all patients of a series of seven Japanese patients with HBV-related FHF (63) and in all five such patients in a study from Israel (64). Conversely, studies from the United States, France, and India, where the prevalence of the precore mutant is low, have demonstrated that this mutation is not necessary for the development of fulminant hepatitis B (69,70,71). Additional HBV mutations involving nucleotides 1762 and 1764 within the precore promoter region, resulting in increased viral replication but similarly impaired HBeAg synthesis, were subsequently described (72,73). An association between such mutations and FHF has been reported in Japanese patients (74). More recently, Aritomi et al. (65) demonstrated a correlation between mutations in the core promoter and precore regions of the HBV genome and severity of HBV infection in Japan, with at least one such mutation present in all 7 patients with HBV-related FHF compared with 4 of 41 (9.8%) patients with self-limited, acute hepatitis B.

Both coinfection with HBV and hepatitis D virus (HDV) and superinfection of chronic HBV carriers with HDV have been associated with development of FHF (75). Seropositivity for anti-HBc IgM was apparent in only 5 of 28 (18%) cases of FHF in Taiwan in which HBV and HDV were implicated as causes (76), suggesting that most patients had superinfection rather than coinfection with HDV. Investigations among Yucpa Indians in Venezuela were the first to disclose the importance of HDV superinfection in an open community as the cause of large outbreaks of FHF in populations with a high preexisting HBV endemicity (77,78). A similar association has been be recognized for previous epidemics of FHF in Brazil and Colombia (78,79,80). Cases of FHF due to dual HBV and HDV infection are becoming less common in southern Europe, consequent to the decreasing prevalence of HDV in recent years (81). Evidence from India suggests a worse outcome in dual HBV- and HDV-infected patients with FHF compared to those having infection with HBV alone (82). Coinfection with HAV and HBV and superinfection with HAV of chronic HBV carriers leading to FHF have also been reported (61,83).

Hepatitis E virus (HEV), an enterally transmitted ribonucleic acid(RNA) virus of the calicivirus group (84) for which no specific antiviral treatment is available, is the most common cause of epidemic hepatitis and FHF in tropical countries such as India and other

developing countries of southeast Asia, accounting for 50% of cases of FHF in a recent series (85,86,87,88). A particularly high prevalence of infection with HEV, along with a high mortality rate, has been documented in pregnant women, especially during the second and third trimesters (85,87). HEV infection has also occasionally been implicated in a relatively small number of sporadic cases of FHF in the West (89).

There is also a striking geographical difference in the prevalence of FHF due to HCV infection. In Japan and Taiwan, HCV positivity has been found in up to 59% of patients with FHF of presumed viral origin and in whom markers for HAV and HBV were negative (90,91,92). Conversely, infection with HCV alone is an uncommon cause of FHF in the West (61,89,93,94,95) and in India (85,87). An exception is the 60% positivity rate for HCV RNA reported in patients of low socioeconomic status and Hispanic ethnicity in Los Angeles (96). There is also evidence of direct hepatitis C transmission to a chimpanzee using serum obtained from an HCV RNA–positive patient with FHF, who developed recurrent disease after OLT (97). Instances of FHF related to HCV have also been reported in Italian patients with chronic HCV infection after the withdrawal of chemotherapy (98), a situation analogous to that seen with chronic HBV infection, as discussed earlier. As in the latter circumstance, levels of HCV viremia were typically low at the time of the fulminant illness. Risk of FHF is increased in HCV and human immunodeficiency virus–coinfected patients treated with highly active antiretroviral therapy (99).

Feray et al. (95) found in a study in France that 8 of 17 (47%) individuals with HBV-related

FHF also had evidence of HCV infection, with the combination of HCV RNA in the liver or serum (including negative-stranded replicative intermediates) but negative anti-HCV indicating acute, replicative HCV infection. Among the eight HCV RNA– and HBsAg-positive cases, five (63%) were anti-HBc IgM–positive, suggesting coinfection with HBV and HCV, while the remaining three individuals (37%) had no detectable anti-HBc IgM and were probably chronic HBV carriers superinfected with HCV. Superinfection with HCV was also apparent in 9/46 (20%) chronic HBsAg carriers with FHF in Taiwan (92). Up to 43% of patients with FHF in India also show dual infection with HEV and HCV (86,87).

A non–A to E presumed hepatitis virus is thought to currently account for 19% of otherwise indeterminant cases of FHF in the United States (100), 14% to 18% in India (85,87), and 16% in Taiwan (101). In this context, the potential of three recently described viruses to cause non–A to E FHF, namely GB virus-C (GBV-C)/hepatitis G virus (HGV), transfusion transmitted virus (TTV), and the SEN virus, warrants consideration. GBV-C/HGV is a globally distributed RNA virus of the Flaviviridae family that is transmitted predominantly by the parenteral route (102,103). Although GBV-C/HGV was found in up to 50% of patients with FHF in earlier series (104,105), more recent data from both the East and West suggest that it is neither a hepatotropic virus nor responsible for FHF, either by itself or in concert with other known hepatitis viruses (101,106,107). TTV, a widely and highly prevalent DNA virus belonging to the circovirus family (108), was initially discovered by representational difference analysis of serum obtained from Japanese patients with posttransfusion non–A to G hepatitis (109). TTV is transmitted by both parenteral and enteral routes (110,111). Recent studies suggest that TTV plays no causal role in the development of FHF (112,113). SEN-V is a novel DNA virus isolated from the plasma of injecting drug users with human immunodeficiency virus infection and is termed after the initials of the patient from whom it was first isolated (114). Testing of patients with FHF of indeterminant etiology in the United States has not revealed an association with this virus (115).

Bone marrow suppression and aplastic anemia are uncommon but well-recognized complications of FHF due to viral hepatitis, especially in children. Recognized associations are with parvovirus B19 and hepatitis viruses A, B, and C. However, the presumed viral infection remains undiagnosed in most cases (116,117,118,119). Bone marrow failure was reported to occur in 8 out of 75 (10.7%) children with FHF admitted to a single center in a recent retrospective series, with the etiology of FHF being parvovirus B19 infection in 2 and non-A, non-B, and non-C hepatitis in 6 (116). Four children with aplastic anemia were treated with antithymocyte globulin (ATG) or antilymphocyte globulin (ALG). Two of these children recovered fully, whereas one developed myelodysplasia and the other, with unresponsive disease, died of septic complications. Of the four patients who did not receive ATG or ALG, three underwent liver transplantation. All four resumed satisfactory granulopoiesis after a median time of 99 days (range 20 to 153 days).

Other Causes

Documentation of cases of FHF due to ingestion of food contaminated with the *Bacillus cereus* emetic toxin, which inhibits hepatic mitochondrial fatty-acid oxidation, raises the possibility that other hitherto poorly categorized mitochondrial toxins may be responsible for many cases currently considered cryptogenic or indeterminant in etiology (6,9,10,11,120,121). Nonacetaminophen drug reactions, mostly idiosyncratic, account for approximately 10% to 15% of cases of FHF in western countries (10). "Ecstasy" (3,4-methylene-dioxymethamphetamine) and other illicit drugs are increasingly recognized causes in our experience (122).

Instances of severe liver damage with the use of antiretroviral agents in patients with human immunodeficiency virus infection are also recognized, either as a direct drug effect or in relation to immune reconstitution in the setting of associated chronic hepatitis B or C viral infections (123,124). Hepatotoxicity due to Chinese herbs is also increasingly seen in western patients.

Other uncommon etiologies of FHF include autoimmune hepatitis, pregnancy-related disorders such as acute fatty liver and the hemolysis elevated liver enzymes and low platelet count (HELLP) syndrome, *Amanita phalloides* poisoning, veno-occlusive disease, acute Budd-Chiari syndrome, hepatic ischemia related to heart failure or septic shock, heatstroke, and Wilson

disease. Specific therapies and interventions to be considered in these disorders include a therapeutic trial of corticosteroid or cyclosporine in autoimmune hepatitis, urgent delivery of the fetus in pregnancy-related etiologies, the use of penicillin and silibinin as antidotes in *Amanita* poisoning, and decompressive vascular shunting (surgical or radiologically achieved) in select patients with veno-occlusive disease and acute Budd-Chiari syndrome. Investigation for an underlying procoagulant disorder is mandatory in this latter disorder. Interventional radiology including not only placement of a transjugular intrahepatic portosystemic shunt (TIPS) but also hepatic venous angioplasty and stenting of the inferior vena cava may have a role in patients with hepatic venous outflow block, depending on the exact clinical context. Treatment of precipitating cardiac dysfunction is necessary in ischemic FHF due to left ventricular failure, along with appropriate antibiotics and vasopressor agents in septic shock. Circulatory collapse with resultant ischemic liver injury also contributes to the pathogenesis of FHF associated with heatstroke.

Recognition of the rare fulminant presentation of Wilson disease, suggested clinically by the presence of hemolysis, splenomegaly, and Kayser-Fleischer rings, is crucial because mortality in those with severe encephalopathy is virtually 100% without urgent OLT. By contrast, survival without transplantation can be achieved with early D-penicillamine treatment in most patients with nonencephalopathic Wilson disease who present acutely with other manifestations of severe hepatic insufficiency, highlighting the importance of early recognition of this disorder (125). Lymphomatous infiltration of the liver is another rare but potentially treatable cause of FHF. Making a specific diagnosis is also important in view of the potential for recurrence of the lymphoma post-OLT (126). Many of the etiologies for which specific therapies may be applied can be established only by liver biopsy, using a transjugular approach in cases with uncontrollable coagulopathy or more than minimal ascites.

Spectrum of Liver Damage

Severe damage to the liver generally has already occurred by the time of presentation (Table 21.3). Histologic examination at this time typically demonstrates confluent necrosis with cell dropout and parenchymal collapse in either a zonal or a nonzonal distribution, especially in cases related to acetaminophen, other drugs and toxins, viruses, and ischemia (127). A considerable number of activated sinusoidal-lining cells, including Kupffer cells, stellate cells, and endothelial cells, may be seen, particularly when cell dropout has commenced in the perivenular region. Increasing evidence suggests that hepatocyte necrosis in FHF results both as a direct consequence of the initiating drug, toxin, viral, or other causes and from the subsequent activation of these various nonparenchymal cells with the release of cytokines (128,129,130). Marked cholestasis, limited to areas of surviving parenchyma, may occur and is not necessarily a poor prognostic sign. Features of venous outflow block, namely sinusoidal dilatation and congestion, are additionally found in cases of FHF due to Budd-Chiari syndrome. Most patients presenting with FHF due to Wilson disease are already cirrhotic (131), although also displaying superimposed parenchymal necrosis and/or collapse, steatosis, moderate portoseptal and parenchymal inflammation, and typically florid ductular proliferation with cholestasis. The presence of orcein-positive granules in a patchy distribution within hepatocytes and macrophages is a distinctive feature. Evidence of CD95-mediated apoptosis has also been reported (132).

Less commonly, microvesicular steatosis in the absence of substantial hepatocyte necrosis or parenchymal loss dominates the histologic picture in FHF, as in cases due to acute fatty liver of pregnancy and with hepatotoxicity related to various mitochondrial toxins, valproic acid, and the tetracyclines. Malignant infiltration of the liver, most often with lymphomatous, leukemic, or metastatic carcinomatous deposits, represents yet

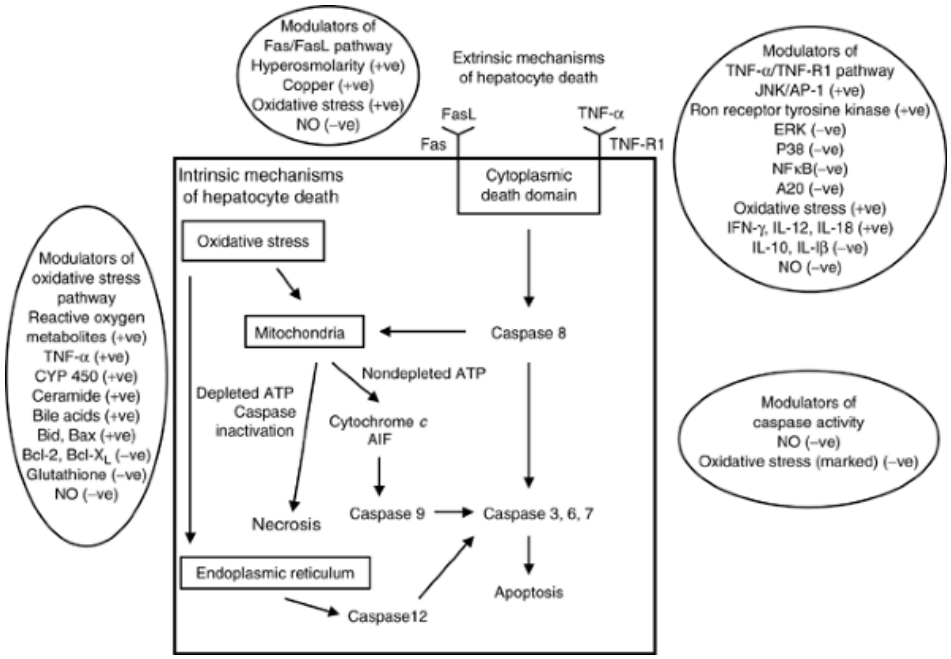
another pathologic process occasionally evident in case of FHF.

Table 21.3. Spectrum of Liver Damage Related to the Etiology of Fulminant Hepatic Failure

Pathology	Etiology
Confluent necrosis (+ activation of nonparenchymal cells)	Drugs (e.g., acetaminophen) Toxins
	Viruses
	Ischemia
Microvesicular steatosis	Acute fatty liver of pregnancy
	Mitochondrial toxins
	Drugs (e.g., valproic acid, tetracyclines)
Malignant infiltration	Lymphoma
	Leukemia
	Metastases

Molecular Mechanisms of Hepatocyte Injury in Fulminant Hepatic Failure

A critical degree of liver cell death, not adequately compensated for by sufficient regeneration of hepatocytes expressing differentiated hepatic functions, is fundamental to the development of FHF. Two pathways of liver cell death exist, namely apoptosis and necrosis. Apoptosis is manifest by nuclear and cytoplasmic shrinkage, without disturbance of cell membrane integrity or liberation of intracellular content. Consequently, secondary inflammation is not a feature. The other pathway of cell death, necrosis, involves depletion of adenosine triphosphate (ATP), with resultant cell swelling and lysis leading to release of cellular content and secondary inflammation (133). At a molecular level, apoptosis occurs as a result of the sequential activation of a series of cysteine proteases, known as caspases. Apoptosis can be triggered by extrinsic or intrinsic mechanisms, the former involving activation of death receptors located on cell membranes and the latter oxidative stress of mitochondria and endoplasmic reticulum (133, 134, 135, 136, 137). The specific caspases involved vary according to the type of proapoptotic stimulus. For example, caspase 8 mediates proapoptotic signal transduction downstream of activated cell surface death receptors, whereas caspase 9 mediates signals that follow oxidative mitochondrial damage. These latter signals augment cell death initiated by activation of death receptors, illustrating important interactions between extrinsic and intrinsic pathways of cell death (133) (Fig. 21.1).



▪ **Figure 21.1** Extrinsic and intrinsic pathways of hepatocellular apoptosis, illustrating interactions of relevance to the development of fulminant hepatic failure. In particular, oxidative stress of mitochondria and endoplasmic reticulum may amplify the apoptotic signals originating from the binding of tumor necrosis factor-α (TNF-α) or Fas ligand (FasL) to their respective "death receptors." TNF-R1, tumor necrosis factor receptor 1; NO, nitric oxide; ATP, adenosine triphosphate; AIF, apoptosis-inducing factor; ERK, extracellular signal-regulated kinase; P38, P38 mitogen activated protein kinase; NFκB, nuclear factor κB; A20, a TNF-inducible protein; IR, ischaemia-reperfusion; IFN-γ, interferon-γ; IL, interleukin; JNK, c-Jun *N*-terminal kinase; AP-1, activating protein-1; Bcl2, Bcl-X_L: members of the Bcl protein family; CYP450, the microsomal cytochrome P450 enzyme system. (Reproduced from Riordan SM, Williams R. Mechanisms of hepatocyte injury, multiorgan failure and prognostic criteria in acute liver failure. *Semin Liver Dis* 2003;23:203–215, with permission.)

A number of causes of FHF have been shown, predominantly in experimental animal models, to induce predominantly one or the other form of liver cell death, such as necrosis in the case of severe

acetaminophen overdose and apoptosis in the case of ischaemia—reperfusion injury and, as alluded to earlier, fulminant Wilson disease (133, 138, 139, 140, 141). Nonetheless, an insult capable of inducing apoptosis may cause cell death by necrosis, particularly if the degree of mitochondrial damage is sufficient to exhaust ATP stores. Processes leading to marked oxidative stress typically cause cell death by necrosis rather than apoptosis, as a consequence of not only the severity of mitochondrial damage but also inhibition of the proapoptotic caspase cascade (133, 142, 143, 144) (Fig. 21.1). Cellular content of the antioxidant, glutathione, and nitric oxide (NO), along with osmolarity and an increasingly recognized number of tyrosine kinases, adapter molecules, transcription factors, cytokines, and chemokines act as important factors that modulate pathways of liver cell death (145). In the clinical setting, intrahepatic expression of interferon-γ (IFN-γ), interleukin-2 (IL-12), and IL-10 has recently been assessed in the explants of 11 patients undergoing transplantation for fulminant hepatitis B (146). A marked induction of the proinflammatory mediators IFN-γ and IL-12 was apparent at both the protein and messenger RNA (mRNA) levels and not counterbalanced by the anti-inflammatory IL-10. A similar pattern was found in five patients with FHF due to other etiologies, including autoimmune hepatitis, ecstasy-related hepatotoxicity, and cryptogenic, implying that imbalance between pro- and anti-inflammatory

cytokine mediators may contribute to the pathogenesis of FHF due to a range of etiologies. Increased circulating levels of IL-6 and IL-8 (147) along with tumor necrosis factor-α (TNF-α), soluble TNF-R1, and IL-10 (148) have also been reported.

Mechanisms Impacting Hepatocellular Regeneration

A variable degree of liver regeneration is evident histologically in most cases of FHF, with the extent of regenerative activity typically more pronounced in hyperacute than subacute categories. Factors responsible for the adequacy, or otherwise, of regeneration in the setting of such severe liver cell loss remain incompletely understood. Nonetheless, increasing evidence points to key roles for a number of cytokines, including TNF-α and IL-6, and growth factors, especially hepatocyte growth factor (HGF). Increased circulating levels of TNF-α and IL-6 have each been described (147,148). These cytokines are key initiators of liver regeneration after partial hepatectomy, although TNF-α–related cell death rather than regeneration predominates in the presence of oxidative stress (149), as discussed in the preceding text. The importance of the IL-6 system in hepatocellular regeneration in FHF has been suggested in a murine model of d-galactosamine—induced liver injury, in which gene therapy with hyper-IL-6, a chimeric protein formed by the fusion of human IL-6 and a truncated form of its soluble receptor that acts as a superagonist, was associated with markedly increased regenerative activity and improved survival (150,151).

Plasma levels of stimulatory HGF and inhibitory transforming growth factor-β (TGF-β) are also elevated in FHF, presumably because of their release from the damaged extracellular matrix (152,153). Increased activity of the fibrinolytic system, responsible for activation of both HGF and TGF-β (154,155), is also evident (156). Nonetheless, toxins that impair HGF-induced DNA synthesis by hepatocytes have been described in plasma of FHF patients (157). Studies of liver tissue from experimental animals and patients with FHF have shown reduced hepatic expression of c-MET, the receptor for HGF (158), raising the possibility that HGF-mediated intracellular signaling may be impaired. Despite this, increased regeneration has been observed in rats with carbon tetrachloride—related FHF treated with exogenous human recombinant HGF (159). Regeneration was also augmented following treatment with anti-TGF-β antibody in such a model (160). The antimicrobial agent, ciprofloxacin, also significantly enhanced hepatic regenerative activity in a rat model of FHF, most likely by blocking hepatocyte membrane receptors for inhibitory γ-aminobutyric acid (161,162).

Impaired regeneration after partial hepatectomy, along with increased susceptibility to acetaminophen-related hepatotoxicity, has recently been documented in peroxisome proliferator—activated receptor-α null mice, possibly as a consequence of altered expression of the genes responsible for cell cycle control, cytokine signaling, and fat metabolism (163,164). The latter may be particularly important because replicating hepatocytes require β-oxidation of fatty acids in the mitochondria for energy (165). Supplementation with free fatty acids and carnitine, the carrier responsible for the transport of fatty acids into mitochondria, augments the rate of regeneration after partial hepatectomy in the rat (166). The role of such supplementation in the FHF setting, in which mitochondria may be profoundly damaged and unable to oxidize fatty acid supplements, has not been assessed but is of potential importance because rapid depletion of fat stores is often seen in this condition, as discussed in the preceding text.

Studies performed in experimental animals after partial hepatectomy demonstrate that hepatic regeneration is also a phosphate-consuming process. Several processes contribute to this, including protein phosphorylation (167), a rapid turnover of intracellular high-energy phosphate (168), and requirement for increased synthesis of phospholipids (169). A substantial fall in serum phosphate values is often seen clinically in patients after partial hepatectomy, presumably

as a result of substrate utilization due to a high degree of regenerative activity (170). Hypophosphatemia is also seen in a substantial proportion of patients with FHF and correlates with a favorable outcome. Conversely, hyperphosphatemia, possibly reflecting impaired hepatocellular regenerative activity, has been identified as a poor prognostic marker (171).

In addition to strategies that enhance liver regeneration to improve outcome in FHF, recent data suggest that strategies to augment the function of surviving hepatocytes may also be of

value. In particular, treatment with lovastatin, a drug that inhibits the p21-Ras—mediated signaling pathway (172), shown in rat hepatocytes to downregulate several liver-specific functions (173), has been reported to improve ammonia metabolism, glucose production, and clotting factor synthesis and substantially reduce the mortality rate in rats subjected to 90% partial hepatectomy (174). Such beneficial effects preceded the initiation of hepatic regeneration, implying that they resulted from an improvement in the functional status of surviving hepatocytes. Notably, the dose of lovastatin used was in the order of 13-fold higher than that recommended in humans for the treatment of hypercholesterolemia, and any impact of such intervention in the clinical setting remains to be evaluated.

Pathophysiology of the Resultant Clinical Syndrome

Hemodynamic Changes

Hemodynamic studies have shown that FHF is characterized by marked splanchnic and systemic arteriolar vasodilatation along with a hyperdynamic circulation and low arteriovenous oxygen content difference. Recent clinical data suggest that elevated levels of IL-6 and IL-8 may contribute to this splanchnic and systemic vasodilatation and the systemic hypotension commonly evident in this syndrome (147). Adrenal insufficiency may contribute to the propensity for systemic hypotension (175), as has been demonstrated in patients with severe sepsis. Studies using the Fick method to determine oxygen transport parameters have suggested that, as in patients with severe sepsis, the ability to extract oxygen at the cellular level is impaired in those with FHF. Consequently, tissue oxygen consumption becomes dependent on oxygen delivery over a wider range of delivery rates than in health (pathologic oxygen supply dependency) (176). The pathogenesis of the microcirculatory disorder leading to impaired tissue oxygen extraction is poorly understood, but production of vasoactive cytokines and endothelial damage caused by generation of oxygen-free radicals may be important. Microcirculatory plugging due to formation of microthrombi as a consequence of activation and consumption of platelets, together with increased adhesion of leukocytes to endothelium, may also be contributory, with blood being shunted through non-nutritive arteriovenous channels (176). Increased activity of the vasodilatory NO–cyclic 3′, 5′-guanosine monophosphate (cGMP) pathway is also evident in FHF (177). However, plasma levels of cGMP were found to have no significant correlation with any hemodynamic or oxygen transport parameter, such that the possible role of this system in the pathogenesis of tissue hypoxia is unclear. Relative hypovolemia secondary to reduced vascular resistance would exacerbate any microcirculatory disturbance unless corrected.

The validity of the concept that tissue oxygen extraction is globally impaired in FHF has recently been called into question by the results of a study in which oxygen consumption was simultaneously calculated by the Fick method and indirect calorimetry (178). Only a poor agreement between the two methods was apparent, with the former consistently underestimating oxygen extraction in comparison to the gas exchange technique. The reproducibility of Fick-derived calculations was less than that of those measured from indirect calorimetry, indicating that in patients with a high cardiac output and small arteriovenous oxygen content difference the dispersion of data attributable to measurement error was greater with the Fick method. Indeed, delivery-dependent global tissue oxygen extraction was reported in less than one third of patients with FHF, reaching the level denoting pathologic oxygen supply dependency in none, when indirect calorimetry is used to derive such data (179).

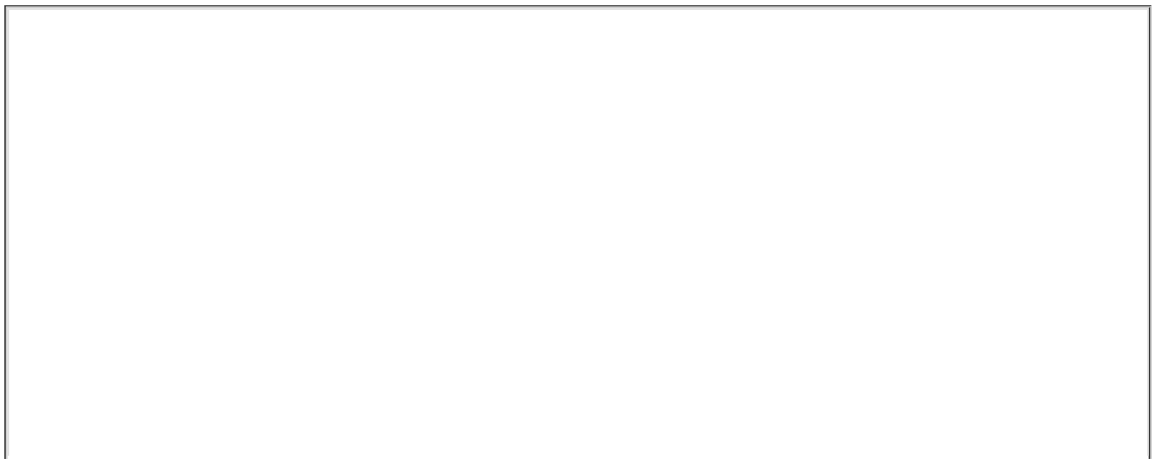
Hepatic Encephalopathy and Cerebral Edema

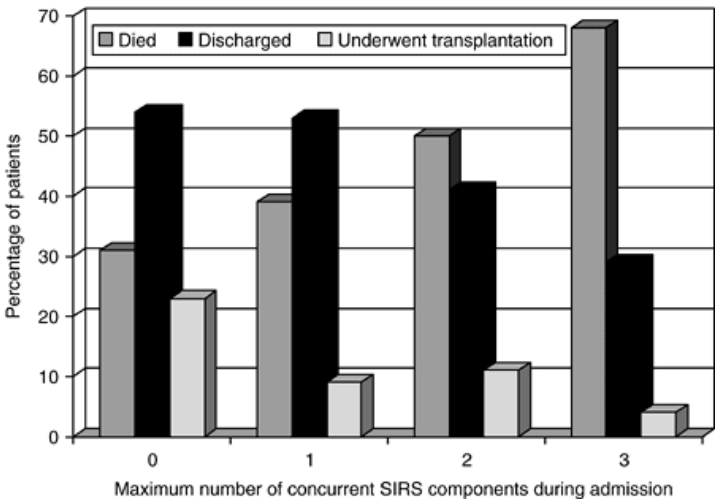
The pathophysiologic mechanisms responsible for encephalopathy in FHF, which is so characteristically rapid in onset and progression, have not been fully elucidated. Accumulation of unmetabolized ammonia; disturbance of central glutamatergic, serotoninergic, and noradrenergic pathways; production of false neurotransmitters; activation of central γ-aminobutyric acid/benzodiazepine receptors; and altered cerebral energy metabolism may be important (180,181,182,183,184,185,186,187). Autoregulation of cerebral blood flow (CBF), through which cerebral perfusion is maintained as a result of reactive dilatation or constriction of cerebral resistance vessels in response to changes in systemic arterial pressure, is impaired or absent in patients with FHF and advanced

encephalopathy grade (188).

The prevalence of severe encephalopathy has fallen in recent years, possibly as a consequence of the recognition that, in those requiring renal support,

continuous hemodiafiltration is preferable to intermittent dialysis, as discussed in the subsequent text. Better control of infection, as discussed later, is probably also an important contributory factor (189). Subclinical epileptiform activity, detectable by continuous electroencephalographic monitoring, is common in patients with FHF and grade III or IV encephalopathy in our experience and this would exacerbate any imbalance between cerebral oxygen demand and supply and precipitate or exacerbate cerebral edema (190). CBF is often increased in FHF, especially at the stage when cerebral edema develops (191). It has been postulated that ammonia is central to the development of cerebral edema in patients with FHF (192), and recent data support an association between arterial ammonia levels and cerebral herniation in this group (193). Ammonia is detoxified in astrocytes to glutamine, the accumulation of which may lead to increased intracellular osmolarity and, in turn, cerebral edema (194,195). Studies performed in the setting of induced hyperammonemia in patients with cirrhosis suggest that the neuropsychiatric effects of ammonia are blunted by the compensatory ability of astrocytes to lose osmolytes such as *myoinositol*, thereby countering the osmotic effects of raised intracellular glutamine levels and reducing the potential for cell swelling (196). This compensatory mechanism may also be important in limiting cerebral edema in patients with FHF. Certainly, increased brain glutamine concentrations have been detected by proton magnetic resonance spectroscopy in this group (197). The local generation of glutamine and glutamate from ammonia, leading to increased neuronal-derived NO levels, has additionally been postulated to be a critical step for the developmente of cerebral edema through the promotion of cerebral hyperemia (198). Nonetheless, the pathogenesis of cerebral hyperemia in FHF remains uncertain, with the recent finding in a rodent model that NO synthase inhibition fails to prevent a rise in CBF associated with hyperammonemia, implying that NO is not the mediator of cerebral hyperemia in this setting (199). Oxidative stress may be important, given preliminary evidence suggesting that zinc protoporphyrin, an inhibitor of oxidative stress—induced activation of heme oxygenase, can prevent increases in CBF and cerebral edema in an experimental hyperammonemic model (200). Exposure to ammonia has been shown to contribute to the propensity for oxidative stress in isolated hepatocytes as a consequence of the production of reactive oxygen species (201), possibly as a result of glutamine-related induction of the mitochondrial permeability transition (202). Accumulation of lactate has also been implicated in the pathogenesis of cerebral edema in FHF (203). Ammonia-induced inhibition of α-ketoglutarate dehydrogenase, resulting in decreased entry of pyruvate into the tricarboxylic acid cycle, likely contributes to the increased lactate production in FHF. The recent finding of a normal hepatic venous pyruvate to lactate ratio suggests that accelerated glycolysis, but not tissue hypoxia, also contributes to the elevated lactate levels (204,205). Increased glycolysis is a known consequence of the systemic inflammatory response syndrome (SIRS) and may be an important mechanism underlying the observation of a significant association between the severity of the SIRS and grade of hepatic encephalopathy in FHF (189). Increased CBF mediated by proinflammatory cytokines such as TNF-α, important mediators of the SIRS, may also be contributory (206).





▪ **Figure 21.2** Outcomes for 887 patients with fulminant hepatic failure and maximum number of concurrent systemic inflammatory response syndrome (SIRS) components during admission. (Reproduced from Rolando N, Wade J, Davalos M, et al. The systemic inflammatory response syndrome in acute liver failure. *Hepatology* 2000;32:734–739, with permission.)

Renal Impairment

Renal failure occurs in approximately 70% of patients with FHF due to acetaminophen and 30% of those with other etiologies (5). Relative hypovolemia due to vasodilatation, microcirculatory disturbance, and acute tubular necrosis related to complicating sepsis are important contributing factors. Direct nephrotoxicity may also play a role in those with FHF related to acetaminophen, especially when the degree of renal impairment is disproportionately severe. The use of nephrotoxic antibiotics may also be a contributory factor.

Susceptibility to Bacterial and Fungal Infection

Patients with FHF are susceptible to infection as a consequence of impaired neutrophil and Kupffer cell phagocytic function, reduced hepatic production of complement, and the requirement for invasive procedures (207). Increased bacterial translocation of gut flora may also be important (208). Culture-proved bacterial infection, most often pneumonia, septicemia, and urinary tract sepsis, occurs in up to 80% of patients (209). The infecting organisms are gram-positive in over 50% of cases. Fungal infection, predominantly with *Candida species*, occurs in over 30%, particularly in the later stages of the clinical syndrome and almost always in association with concurrent bacterial sepsis. Approximately one third of patients with sepsis remain afebrile with a normal white cell count, highlighting the need for close vigilance and a high index of suspicion.

An analysis of 887 patients with FHF admitted to a single center over an 11-year period found significant associations between infection, severity of SIRS (temperature >38°C or <36°C, heart rate >90 beats/minute, tachypnea >20 breaths/minute or Paco2 <4.3 kPa, white cell count >12 × 109/L or <4 × 109/L, or the presence of >10% immature neutrophils), and progressive encephalopathy, reducing the chance of OLT and conferring a poor prognosis (189) (Fig. 21.2). Sepsis is a major cause of the SIRS in FHF, and sepsis-related oxidative stress (210) has been shown in rodents to both promote hepatocellular necrosis and inhibit liver cell regeneration (211), upon which spontaneous recovery ultimately depends.

Nutritional Disturbance

Energy requirements in FHF are increased by up to 60% and are further elevated by complicating infection. Mean energy expenditure has been estimated to be 4.05 kJ/kg per hour. Despite the reduction in functioning liver mass, the metabolic rate is substantially increased (212), in keeping with the marked SIRS that typically accompanies this syndrome. Harris-Benedict predictions are unreliable for estimating energy expenditure in the FHF setting (212). Rapid deterioration in nutritional status, with depletion of muscle and fat stores, is often seen. Impairment of glycogen storage and reduced capacity for gluconeogenesis result in increased breakdown of adipose tissue and muscle consequent to the use of fat and protein as alternative fuel sources (213). However, the predominant factor responsible for the exaggerated whole body protein degradation is likely reduced hepatic synthesis of insulin-like growth factor-1 (214). Hypoglycemia occurs early in the course of FHF; hypophosphatemia, hypokalemia, and hypomagnesemia are also common, especially in patients who maintain an adequate urine output. As is well described in chronic liver disease, impaired peripheral uptake of glucose consequent to insulin resistance has been documented early in the course of FHF, with insulin sensitivity typically being restored by 2 weeks in patients who survive (215).

Coagulopathy and Bleeding Diathesis

Several mechanisms contribute to the coagulopathy associated with FHF, including reduced hepatic synthesis of clotting and anticlotting factors together with consumption of clotting factors and platelets due to disseminated intravascular coagulation (DIC) (216). Although evidence of the latter may be obtained from moderately raised levels of fibrinogen degradation products in a substantial proportion of patients, as has been well demonstrated in experimental animal models, DIC is usually not severe except in acute fatty liver and other pregnancy-related etiologies. The platelet count generally falls progressively day-by-day and is a good marker of disease stage and prognosis. In addition to thrombocytopenia, qualitative platelet defects including increased adhesiveness and impaired aggregation have been described. The degree of prolongation of the prothrombin time is closely related to the severity of liver damage, whereas factor V has the shortest half-life and is, theoretically, the most sensitive index of impaired clotting factor synthesis. Deficiencies of anticlotting factors, such as protein C and antithrombin III, may result in thrombosis of dialysis circuits, despite other manifestations of a bleeding diathesis.

Supportive Management

Management in a specialized liver intensive care unit is mandatory for all patients with FHF and more than grade I hepatic encephalopathy. Supportive medical interventions, based on an understanding of underlying pathophysiology, are aimed at maintaining

hemodynamic, cerebral, and renal function; reversing metabolic derangements; preventing or treating complicating bacterial and/or fungal infection; preventing stress ulceration of gastric mucosa; and, where appropriate, treating coagulopathy.

Hemodynamic

Interventions to achieve or maintain hemodynamic stability are aimed at optimizing tissue oxygen delivery and consumption parameters. Substantial volumes of colloid, as well as crystalloid, may be required to attain an adequate cardiovascular filling pressure (i.e., pulmonary capillary wedge pressure 8 to 14 mm Hg), given the often profound vasodilatation. In studies performed at King's College Hospital using the Fick method and reported in 1991, *N-acetylcysteine infusion* was shown to facilitate improved hemodynamic stability in association with mean 46% and 29% increases in global tissue oxygen consumption after 30 minutes of its administration in patients with acetaminophen and other etiologies of FHF, respectively; prostacyclin infusion also improved oxygen delivery and consumption, whereas combined infusions led to a significant increase in oxygen delivery but not consumption compared to infusion of N-acetylcysteine alone (217). A more variable systemic hemodynamic response to N-acetylcysteine was subsequently reported, with clear separation of responders and nonresponders. Overall, a small (6%) early improvement in tissue oxygen consumption that was not sustained throughout a 5-hour period of monitoring was recorded (179). Results of a multicenter study currently under way in the United States are awaited with interest.

Vasopressor agents are indicated if the mean arterial pressure (MAP) is <60 mm Hg despite adequate intravascular volume. Most experience is with the use of epinephrine and norepinephrine. Oxygen consumption may fall with the use of these agents despite the increased arterial pressure, as a result of reduction in oxygen delivery and extraction rates. The latter may be prevented by concurrent use of prostacyclin (218). Recent data supporting the use of vasopressin in severe vasodilatory shock syndromes (219,220), in which baroreflex-mediated secretion of this agent becomes impaired (221), raise the possibility that vasopressin infusion may also be of value as an alternative to epinephrine or norepinephrine for patients with marked vasodilatation and refractory hypotension. The vasopressin prodrug, terlipressin, is less likely to cause myocardial ischemia than vasopressin but should be used with caution in those with grade IV hepatic encephalopathy and raised intracranial pressure (ICP) in view of recent evidence that it may exacerbate cerebral hyperemia and intracranial hypertension (222).

Hepatic Encephalopathy and Cerebral Edema

Neurologic support is aimed at optimizing CBF, cerebral perfusion pressure (CPP) and oxygen consumption, along with preventing or treating cerebral edema. Patients should be nursed in the 20- to 30-degrees head-up position. Elective mechanical ventilation, with sedation and paralysis using agents such as propofol and atracurium, along with minimization of endotracheal suctioning, patient turning, and other tactile stimulation, prevents surges in ICP, which may provoke or exacerbate cerebral edema. Treatment with N-acetylcysteine has been reported in one study to increase CBF and cerebral oxygen consumption, with a fall in anaerobic metabolism; infusion of prostacyclin also improves CBF (223). Extradural pressure monitoring, with sufficient clotting factor support to achieve an international normalized ratio of 2 or less and platelet transfusions to achieve a count of 50 × 109/L or more at the time of insertion of the transducer, is of value in guiding further therapy in ventilated patients with grade III or IV encephalopathy. It is particularly valuable during OLT because the increase in CBF that occurs with reperfusion may be deleterious, especially in those with defective autoregulation (224). Such monitoring does carry some risk of hemorrhage at the time of insertion of the transducer or later, and the transducer should be removed no longer than 5 days after insertion in view of the risk of infection. A fall in CPP, the difference between MAP and ICP, to less than 50 mm Hg because of arterial hypotension should be managed with vasopressor agents, provided intravascular volume status is adequate. When CPP is reduced as a consequence of an increase in ICP, or when the latter exceeds 25 mm Hg, bolus intravenous injection of mannitol (0.5 g/kg body weight) is first-line treatment (223). Renal replacement therapy, aiming to remove two to three times the volume of infused mannitol, is required in patients in oliguric renal failure. Repeated doses may be given as necessary, provided the plasma osmolarity does not exceed 320 mOsmol/L. Thiopentone may be beneficial in cases of intractable cerebral edema, although it may result in further hemodynamic instability.

Considerable interest at present centers on the relatively simple procedure of induction of moderate hypothermia (32°C to 33°C), which, as in experimental animals, may be beneficial in both adults and infants with uncontrolled increases in ICP through reductions in CBF, cerebral metabolism, and glutamine synthesis (225,226,227,228,229). Lowering of core body temperature to 32°C to 33°C using cooling blankets for periods ranging from 10 to 118 hours led to a significant reduction in CBF in 14 patients with FHF awaiting OLT and with increased ICP refractory to standard medical therapy in several recent reports (225,228,229). Significant

reduction in ICP and improved CPP were documented, along with reduced requirement for norepinephrine support (225,229) (Table 21.4). Thirteen of 14 liver transplantation candidates were successfully maintained until the time of OLT (229). Stimulation of the detoxification of ammonia in skeletal muscle with ornithine aspartate (230,231) and antagonism of postsynaptic glutamate-*N*-methyl-D-aspartate (NMDA) receptors with memantine (232) may also have therapeutic potential, either alone or in combination.

Table 21.4. Changes in Systemic and Cerebral Hemodynamics with Induction of Moderate Hypothermia (32°C To 33°C) in Patients with Fulminant Hepatic Failure

Having Raised Intracranial Pressure Unresponsive to Mannitol and Hemofiltration

	Before cooling mean (SEM)	4 hour after cooling mean (SEM)	10–24 hour after cooling mean (SEM)
Systemic vascular resistance (dyne s/cm^5)	503.8 ± 41	671 ± 48.4[a]	716.8 ± 43.3[b]
Mean arterial pressure (mm Hg)	76 ± 3.6	82.8 ± 2.2[b]	77 ± 1.8[c]
Intracerebral pressure (mm Hg)	36.5 ± 2.7	16.3 ± 0.7[c]	16.8 ± 1.5[c]
Cerebral blood flow (mL/100 g min)	78.2 ± 9.7	46.5 ± 3.8[c]	44.0 ± 1.9[c]
Cerebral perfusion pressure (mm Hg)	40.1 ± 2.9	66.4 ± 2.8[c]	67.2 ± 2.8[c]

[a]*P* <0.05 compared to precooling.
[b]*P* <0.01 compared to precooling.
[c]*P* <0.001 compared to precooling.
SEM, standard error of the mean.
Adapted from Jalan R, Olde Damink SWM, Deutz NEP, et al. Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. *Gastroenterology* 2004;127:1338–1346, with permission.

Prophylactic phenytoin has been found in one study to reduce the incidence of subclinical epilepsy and associated cerebral edema, with evidence of the latter at autopsy in 22% of treated patients compared to 70% of untreated counterparts (190). Seizure activity despite phenytoin treatment may respond to diazepam, although hypotension related to the use of this drug must be quickly reversed, and benzodiazepines should otherwise be avoided in view of the possible role of activation of central γ-aminobutyric acid/benzodiazepine receptors in the pathogenesis of encephalopathy. The somewhat dramatic procedure of total hepatectomy with temporary portocaval shunt is worth considering during the period between organ retrieval and transplantation in those awaiting OLT. In cases with refractory intracranial hypertension, both cerebral and systemic hemodynamics can be substantially improved (233). Hyperventilation reduces CBF and is appropriate in the subgroup with cerebral hyperemia, as reflected by increased reverse jugular venous oxygen saturation and elevated ICP. A reverse jugular venous oxygen saturation of 55% to 75% and an arteriojugular venous lactate difference of 35 mmol/L or less suggest an adequate CPP.

Renal Failure

Ensuring adequate intravascular volume status and treatment of any complicating infection are important measures to prevent or treat established renal impairment. In patients with FHF related to acetaminophen, the incidence of renal failure requiring dialysis was reported to be reduced by *N*-acetylcysteine infusion in one study, even after severe liver damage had occurred (50). Indications for renal replacement therapy in FHF include uncontrolled acidosis, hyperkalemia, fluid overload, and oliguria associated with either a serum creatinine level >300 μmol/L or cerebral edema requiring treatment with mannitol. Continuous veno–veno hemodiafiltration is preferable to intermittent hemodialysis because complicating hypotension

with the latter results in a fall in CPP that may exacerbate or precipitate cerebral edema (234).

Susceptibility to Infection

Prophylactic parenteral broad-spectrum antibiotic regimens, such as ceftazidime plus flucloxacillin or piperacillin plus tazobactam, combined with enteral amphotericin B and vaginal clotrimazole in one study reduced the incidence of infection from 80% to 20%. The latter approach was as effective as the more intensive enteral decontamination regimens (235). Proved bacterial infection should be treated according to in vitro sensitivities, whereas invasive fungal infection requires parenteral treatment with an appropriate antifungal agent. In the absence of a positive isolate, the possibility of fungal infection should be considered in the settings of a fever unresponsive to broad-spectrum antibiotics, leukocytosis, or deterioration in neurologic status after initial improvement, especially in the presence of renal failure. Granulocyte colony-stimulating factor improves neutrophil function both in vitro and in vivo in FHF (236,237) and may have a future role in preventing or treating infection in this group.

Nutrition

Caloric requirements in the order of 35 to 50 kcal/kg daily are required to meet the resting metabolic demand. Protein intakes in excess of 1g/kg daily are necessary to maintain nitrogen balance (238). Up to

50% of nonprotein calories should be delivered as lipid. Hypoglycemia, hypophosphatemia, hypokalemia, and hypomagnesemia require aggressive replacement. Enteral nutrition is considered preferable to parenteral in view of reports of maintained integrity of gut mucosa and reduced rates of bacterial translocation and sepsis in experimental animals (208).

Coagulopathy and Bleeding Diathesis

Because the prothrombin time is an important prognostic variable, infusion of fresh frozen plasma is indicated only for bleeding or at the time of invasive procedures, such as insertion of ICP monitors, as discussed earlier. Platelet transfusions are required in the latter circumstances if the count is less than 50×10^9/L and if less than 20×10^9/L platelets should be given prophylactically. Recent data in a small number of patients with FHF suggest that plasma infusion in combination with recombinant activated factor VII may be advantageous (239). The risk of bleeding from stress ulceration of gastric mucosa is reduced by the prophylactic use of sucralfate. This agent is preferable to antisecretory drugs, which predispose to gastric bacterial overgrowth and nosocomial pneumonia. Endoscopic treatment of bleeding varices, which can develop acutely in FHF, or of uncontrolled hemorrhage from stress ulceration may be required. Use of low-dose heparin or prostacyclin is generally required to prevent thrombosis of dialysis circuits, despite other manifestations of a bleeding diathesis.

Selection Criteria for Liver Transplantation

Reliable, clinically applicable, early markers of prognosis are required to accurately stratify risk in individual patients and ensure that those who are highly likely to die without OLT are listed as early as possible, thereby maximizing the time for a donor organ to be procured, while at the same time sparing unnecessary transplantation in other patients, in whom spontaneous recovery will otherwise occur.

Table 21.5. King's College Selection Criteria for Orthotopic Liver Transplantation According to Etiology of Fulminant Hepatic Failure

Etiology	Selection criteria for OLT
Acetaminophen	Arterial pH <7.30 despite normal intravascular filling

	pressures (irrespective of grade of encephalopathy)
	OR
	Prothrombin time >100 s + serum creatinine level >300 μmol/L in patients with grade III or IV encephalopathy
Nonacetaminophen	Prothrombin time >100 s (irrespective of grade of encephalopathy)
	OR
	Any three of the following (irrespective of grade of encephalopathy): Non-A, non-B hepatitis (cryptogenic), halothane hepatitis, or other drug toxicity Age <10 y or >40 y Jaundice-to-encephalopathy interval >7 d Prothrombin time >50 s Serum bilirubin >300 μmol/L

OLT, orthotopic liver transplantation.
Reproduced from reference O'Grady JG, Alexander GJM, Hayllar KM, et al. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* 1989;97:439–445, with permission.

King's College Criteria

Selection criteria for OLT in the setting of FHF are not currently standardized worldwide. Nonetheless, those formulated at the King's College Hospital, following retrospective, multivariate analysis of possible prognostic factors in 588 patients treated medically between 1973 and 1985 (4), are widely applied (Table 21.5). The original assessment of the accuracy of these indicators was based on the consideration of outcome, again retrospective, in a further consecutive series of patients managed during 1986 and 1987, including 121 with FHF due to acetaminophen and 54 with other etiologies. Positive predictive values for death (the proportion of patients fulfilling criteria who died) in the acetaminophen and nonacetaminophen groups were 84% and 98%, respectively, whereas negative predictive values (NPVs) (the proportion of patients not fulfilling criteria who survived) were 86% and 82%, respectively. Predictive accuracies (the proportions of all patients in whom outcome was correctly predicted) were 85% and 94%, respectively.

Several subsequent reports from other centers using the King's College criteria indicated that, although fulfillment of criteria carries a poor prognosis for spontaneous survival, lack of fulfillment carries a less favorable outlook than originally suggested (7,9), leading to much uncertainty about which patients can be managed without listing for urgent transplantation (Table 21.6). Shakil et al. (7) reported retrospectively on the Pittsburgh experience with these criteria in 177 patients over a 13-year period up to 1995, the first such assessment to be performed in the United States, adding

to two previously published validation studies from the United Kingdom and Europe (9,240). Approximately 50% of the patients in the Pittsburgh series underwent OLT a median 3 days after admission. The spontaneous survival rate was 14%, while almost three times as many patients died a median 6 days after admission without undergoing OLT. Patients who died

without OLT were significantly more likely than those who survived spontaneously to have grade III or IV encephalopathy at the time of admission, in keeping with previous reports from the United Kingdom and United States (4,7,9). Most patients who died without OLT had been considered transplantation candidates, at least early in their clinical course, compatible with the experience in the United Kingdom, where approximately 35% of those initially suitable for OLT rapidly developed contraindications such as uncontrolled cerebral edema, sepsis, severe hemodynamic disturbance, and multiorgan failure and became unsuited for transplantation (241).

Table 21.6. Validation of the King's College Prognostic Criteria in Patients with Fulminant Hepatic Failure

Criteria	PPV (%)	NPV (%)	PA (%)
FULMINANT HEPATIC FAILURE DUE TO ACETAMINOPHEN			
O'Grady et al. 1989 (London) (4)			
Arterial pH <7.3	95	78	81
PT >100 s, serum creatinine level >300 μmol/L, and grade III or IV encephalopathy	67	86	83
Overall	84	86	85
Anand et al. 1997 (Birmingham) (9)			
Arterial pH <7.3	77	64	70
PT >100 s, serum creatinine level >300 μmol/L, and grade III or IV encephalopathy	79	72	73
Overall	73	71	72
Shakil et al. 2000 (Pittsburgh) (7)			
Arterial pH <7.3	69	80	72
INR >6.5, serum creatinine level >300 μmol/L, and grade III or IV encephalopathy	100	79	86
Bernal et al. 2002 (London) (241)			
Overall	80	94	—
Schmidt and Dalhoff, 2002 (Denmark) (171)			

Overall	80	93	92
FULMINANT HEPATIC FAILURE DUE TO NONACETAMINOPHEN CAUSES			
O'Grady et al. 1989 (London) (4)			
PT >100 s	100	26	46
Any three of five variables[a]	96	82	92
Overall	98	82	94
Pauwels et al. 1993 (Paris) (240)			
Overall			
At admission	96	50	80
48 h before death	89	47	79
Anand et al. 1997 (Birmingham) (9)			
PT >100 s	100	37	52
Any three of five variables[a]	65	17	52
Overall	68	25	61
Shakil et al. 2000 (Pittsburgh) (7)			
PT >100 s	98	50	79
Any 3 of 5 variables[a]	91	42	74

[a]PT >50 s; jaundice-to-encephalopathy time >7 d; non-A, non-B hepatitis or drug-induced etiology; age <10 y or >40 y; serum bilirubin >300 μmol/L.
PPV, positive predictive value; NPV, negative predictive value; PA, predictive accuracy; PT, prothrombin time; INR, international normalized ratio. From Riordan SM, Williams R. Use and validation of selection criteria for liver transplantation in acute liver failure. *Liver Transpl* 2000;6:70–173, with permission.

The Pittsburgh analysis found that the King's College prognostic criteria carry acceptably high positive predictive values for death in patients with FHF due to both acetaminophen and other causes. NPVs in the acetaminophen group were also acceptably high in the Pittsburgh series, comparable to those documented in the original report. However, the NPV in

the nonacetaminophen group was found to be seriously lacking, especially in the case of those in the nonacetaminophen group, a finding in accord with that reported previously in 81 patients managed at Hôpital Saint-Antoine, Paris, between 1978 and 1988, and in 145 patients treated at Queen Elizabeth Hospital, Birmingham, between 1990 and 1994 (9,240). Overall predictive accuracies were somewhat reduced in each of these series in comparison to the original assessment (Table 21.6).

There is a need, therefore, to include other factors in the King's College criteria of prognostic significance. Anand et al. (9) identified peak white cell count and hyperkalemia as additional, independent indicators of poor prognosis in acetaminophen-related FHF. Any practical significance in terms of identifying patients whose prognosis would be improved by emergency OLT would likely be limited by the fact that such abnormalities often occur relatively late in the clinical course and in the setting of contraindications such as sepsis and hemodynamic disturbance. In the nonacetaminophen group, NPV was marginally improved without compromising positive predictive value by reducing the cutoff for prothrombin time from 100 to 75 seconds.

The King's College criteria were recently reevaluated in another cohort of patients with FHF due to acetaminophen managed at that center (242). The positive predictive value was 80% compared to 84% in the original series, whereas the NPV was 94%, even higher than the 86% found in the original series, and in keeping with the figure of 93% in a recent series from Denmark (171). With additional consideration of blood lactate levels (a postresuscitation arterial blood lactate level >3.0 mmol/L or either a postresuscitation or an "early" value >3.5 mmol/L), NPVs were 97% and 99%, respectively. Positive predictive values fell to 79% and 74%, respectively. Additional consideration of blood lactate levels modestly improved the NPV, but positive predictive value remained higher with the initial King's College criteria alone (Table 21.6). Patients with a poor outcome were identified earlier when blood lactate levels were taken into consideration (242).

Other Prognostic Criteria

Alternate prognostic indices have been proposed. In a series of 58 patients with acute viral hepatitis, mostly due to HBV infection, who did not undergo transplantation, managed between 1986 and 1990, Bernuau et al. (243) in Clichy found that criteria based on the presence of coma or confusion in association with reduced factor V levels carried positive predictive values and NPVs for death of 82% and 98%, respectively. Clinically apparent encephalopathy was present on admission or subsequently in most, but not all, patients. However, a subsequently reported French study of 81 patients with encephalopathy and nonacetaminophen-related FHF, mostly due to acute viral hepatitis B as in the Clichy series, found a substantially lower ability of the Clichy criteria to correctly identify patients who will survive without OLT (240). Furthermore, the Clichy criteria performed less well in this regard than the King's College criteria when both sets of indicators were applied to the same nonacetaminophen study population, with NPV of 28% and 50%, respectively, on admission, and 36% and 47%, respectively, when reevaluated 48 hours before death (240), a time chosen to approximate the mean waiting time for a donor liver in some contemporary European transplantation series. By contrast, positive predictive values of the two sets of criteria were comparably high (240).

Nevens et al. (244), in a Belgian series of 28 patients with nonacetaminophen-related FHF, found that the overall predictive accuracy was modestly increased when both sets of criteria were considered in combination, although the ability to identify patients who will recover spontaneously remained low even in this circumstance. In the only reported comparative assessment of the two sets of criteria in acetaminophen-related FHF, Izumi et al. (245) in a series of 81 patients found that the Clichy criteria performed less well, with lower positive predictive value (49% vs. 92%) and predictive accuracy (56% vs. 83%). The NPV was, however, acceptably high.

Taken together, these findings suggest that patients with either acetaminophen- or nonacetaminophen-related FHF who fulfill the King's College criteria and those with nonacetaminophen etiologies meeting the Clichy criteria should be listed for urgent OLT, with the exception of nonacidotic acetaminophen patients, when the encephalopathy grade is not

advanced. Patients not fulfilling prognostic criteria, especially with FHF unrelated to acetaminophen, should still be considered for OLT and excluded only if serial assessments indicate spontaneous recovery. This issue will likely become even more difficult if the use of temporary liver support based on extracorporeal perfusion or transplantation of hepatocytes or stem cells, as discussed in the subsequent text, is proved to be of benefit.

Factor VIII/V ratios; serial prothrombin times; assessment of liver size on computed tomography scanning; liver histology; the Acute Physiology and Chronic Health Evaluation (APACHE) score; sensory-evoked potentials; serum levels of Gc-globulin (vitamin D–binding protein), which is an important liver-derived component of the extracellular actin-scavenging system; and the severity of the SIRS have alternatively been proposed as possible prognostic indices in FHF, with varying degrees of applicability and reports of efficacy (246). In the tropical population

of India, in which viral hepatitis is the most common cause of FHF, older age, cerebral edema, and degree of prolongation of prothrombin time have been identified as factors indicative of poor prognosis. As already referred to, fulminant presentations (with encephalopathy) of Wilson disease and Budd-Chiari syndrome in association with extensive hepatocellular necrosis are generally considered to represent indications for urgent OLT. Primary myeloproliferative disorders responsible for the Budd-Chiari syndrome should not be considered a contraindication to this intervention (246).

The possible prognostic value of circulating and intrahepatic cytokine levels has been the subject of several recent reports. In a report from the United Kingdom, circulating levels of both IL-6 and IL-8, but not TNF-α, were found to be significantly higher in patients who subsequently died than in those who survived (147). Lack of correlation with degree of liver failure, as reflected by prothrombin time, serum bilirubin level, or degree of hepatic encephalopathy, suggested that these parameters reflected not the severity of FHF per se but rather complications such as circulatory disturbance and resultant extrahepatic multiorgan failure. Indeed, the hemodynamic instability of which they are reflective would preclude OLT. A Japanese study found that, at hospital admission, circulating levels of TNF-α and IL-10, but not IL-6, were significantly higher in patients who died than in those who survived. In keeping with the United Kingdom experience, circulating levels of these cytokines did not correlate significantly with the degree of liver injury, as reflected by serum transaminase values (148). A German series found no significant correlations between intrahepatic levels of IL-12, IFN-γ, or IL-10, at either protein or mRNA levels, and jaundice-to-encephalopathy time, encephalopathy grade, requirement for inotrope support, serum bilirubin level, prothrombin time, or APACHE II score (146).

As alluded to earlier, hyperphosphatemia, possibly as a consequence of renal impairment and lack of substrate utilization due to blunted hepatic regenerative activity, has recently been reported to be an early predictor of poor outcome in severe acetaminophen-related liver injury (171). In a series of 125 patients, including 30 with hepatic encephalopathy, a threshold phosphate concentration of 1.2 mmol/L or above at 48 to 96 hours after overdose had higher sensitivity, predictive accuracy, and positive predictive values and NPVs for death than the King's College criteria (89% vs. 67%, 98% vs. 92%, 100% vs. 80%, and 98% vs. 93%, respectively). Specificity was 100%. Consideration of the King's College criteria in combination with the phosphate level led to improvement in sensitivity to 94%. As with consideration of blood lactate levels (242), patients with a poor outcome were identified substantially earlier using the phosphate criteria (median 1 hour after referral) than with the King's College guidelines (median 12 hours).

Adrenal insufficiency has also recently been shown to correlate with outcome. Patients who did not survive to discharge from the intensive care unit or underwent liver transplantation had significantly lower increment and peak cortisol levels after stimulation with tetracosactide (Synacthen) than those who survived. Higher incidences of subnormal increment and peak cortisol levels were found in nonsurvivors (55%) than in survivors (21%) (175). Nonetheless, the relative lack of both sensitivity and specificity limits the usefulness of these parameters for prognostic modeling.

Trends in Transplantation Activity

Data from the National Transplant Database of the United Kingdom and Ireland indicate that the number of patients with acetaminophen-induced FHF who were listed for super-urgent OLT increased by more than 75% during the period from 1995 to 1998, accounting for 40% of all super-urgent listings and 38% of all super-urgent transplantations in the 12 months to August 1998. In more recent years, however, the numbers have fallen progressively with the proportion of patients listed and undergoing transplantation in 2001/2002 reducing to 53% and 56% of their 1997/1998 values, respectively. As a corollary, the number of patients listed and undergoing transplantation for FHF of other etiologies has increased, including a more than 50% increase over the last few years in the number of patients listed for acute graft dysfunction after transplantation (247), presumably reflecting a greater use of marginal liver grafts. The number of patients undergoing transplantation for FHF because of non–A to E hepatitis has also increased over this time (247) as a direct consequence of greater organ availability for super-urgent transplantation consequent to the reduction in acetaminophen-related procedures rather than an increase in the prevalence of FHF due to seronegative hepatitis. Priority listing within the super-urgent category in the United Kingdom and Ireland offers the possibility of ABO-matched grafts being available within 24 to 72 hours.

Recent experience from the United States is that 66% of patients with FHF listed for transplantation received a graft after a median waiting time of 3 days and 18% on the waiting list died after a median 5 days. These findings indicate that lack of early donor organ availability remains a crucial factor limiting survival (248). As in the United Kingdom, a relatively low percentage of acetaminophen-related cases compared to those with FHF due to other etiologies underwent transplantation because of the relatively high spontaneous survival rate (248).

Outcome of Transplantation

The most recent analysis from the European Liver Transplant Registry (ELTR) (Hôpital Paul Brousse, Villejuif, France) documents 1-, 5-, and 10-year survival rates of 63%, 59%, and 54%, respectively, after cadaveric OLT for FHF. The plateau in mortality rate after the first 3 months reflects the fact that patients undergoing transplantation are critically ill before the procedure and often undergo a complicated early postoperative period. Survival is substantially reduced in patients with sepsis and multiorgan failure before OLT (249). Nonetheless, ELTR data indicate that those patients who survive beyond the first 3 months follow a survival curve comparable to that seen after elective OLT performed for cirrhosis. A center workload of fewer than 25 transplantations per year and fewer than 20 split liver grafts per year were among the risk factors for poor outcome after liver transplantation (250). In the best centers, outcome with split liver grafts is comparable to that after the use of full-size organs (251).

An important issue is the potential for viral-related cases of FHF to recur post-transplantation, especially under the influence of immunosuppression. Patients undergoing transplantation for fulminant HBV infection have a significantly reduced rate of hepatitis B recurrence than those undergoing transplantation for HBV-related cirrhosis (252). This presumably relates to the high prevalence of viral clearance before the procedure. Recurrent HBV-related FHF is distinctly uncommon. Graft damage due to recurrent HAV and other viral infections, including those described with Toga-like particles, is occasionally seen after OLT for fulminant disease (253). Although some degree of hepatitis C recurrence is almost universal after OLT for cirrhosis, there are little data in the FHF setting. Recurrence of hepatitis E has not been observed in the small number of patients undergoing transplantation for fulminant HEV infection to date.

A retrospective comparison of costs and cost-effectiveness of OLT performed for FHF and cirrhosis was recently reported (254). Costs up to 1 year were estimated to be 107,675 for the cirrhosis cases and 90,792 for those undergoing transplantation for FHF. OLT was considered less cost-effective when performed for FHF than for cirrhosis on account of the lower 1-year post-OLT survival in the former group.

Auxiliary Partial Transplantation

The use of auxiliary partial OLT is based on the undoubted potential for spontaneous liver

regeneration in FHF. This technique involves the removal of the left- or right-lobe segments of the diseased liver and their replacement by the equivalent segments of the donor organ. Right-lobe transplantation is generally used in adults, while a left lobe harvested from an adult graft may suffice for a child. Results published from the European Auxiliary Liver Transplant (EURALT) co-operative Study (255) show a survival rate comparable to that of conventional OLT and that immunosuppressive therapy could be withdrawn, leading to graft atrophy, in 65% of patients surviving at 1 year, in whom adequate regeneration of the native liver had occurred. Similarly, full regeneration of the native liver was documented in 8 of 11 (74%) surviving patients in a series of 17 auxiliary partial OLT recipients from two French centers (256). Immunosuppressive treatment was subsequently withdrawn or the dosage tapered in seven of these eight patients.

Differing results have been reported by Azoulay et al. (257), who recently compared auxiliary partial OLT with standard whole-liver transplantation in a consecutive series of 49 patients undergoing transplantation for fulminant or subfulminant hepatitis at another French center. OLT was performed in 37 patients and auxiliary partial OLT in 12. Each patient treated with auxiliary partial OLT was matched to two patients undergoing OLT according to age, coma grade, etiology of liver failure, and clinical course. Although 1-year patient survival was identical (66%) in the two groups, the complication rate was higher in the auxiliary partial OLT recipients. In particular, patients undergoing the auxiliary procedure experienced significantly more technical complications (1 ± 1.3 vs. 0.3 ± 0.5), episodes of bacteremia, requirement for retransplantation (3/12 vs. 0/24), and neurologic sequelae or brain death (4/12 vs. 2/24). Only in 2 of 12 auxiliary partial OLT recipients (17%) was the procedure a complete success, as reflected by the ability to withdraw immunosuppression and patient survival.

Living-Related Transplantation

In parts of the world where cadaveric organ donation is not widely accepted, recourse has been made to the living-related donor procedure, especially in children. The 1-year survival rates after living-related OLT for FHF in a total of 35 pediatric cases in three reported series (258,259,260), using left lobe and left lateral segments, ranged from 59% to 90%. Living-related left lobe, right lobe, and extended right lobe OLT has also recently been used successfully in cases of adults with FHF. The 1-year survival rate in the largest reported series of 53 adult patients in Japan was 75% (261). Pioneering work in Hong Kong has demonstrated that a graft in excess of 40% of standard liver volume is often required to reverse the severe metabolic derangements that occur in FHF (262,263). This can translate to the requirement for an extended right lobe graft for adult recipients, although

few centers go as far as this. At least some degree of live donor graft injury is common in small-for-size adult recipients. In particular, patients receiving a right lobe graft less than 40% of standard liver weight often develop transient portal hypertension after reperfusion, accompanied by intragraft upregulation of endothelin-1 and ultrastructural evidence of sinusoidal damage (264). Reduction in portal blood flow by portocaval shunting and splenic artery ligation may alleviate this "small-for-size" phenomenon and even allow the use of the left lobe for transplantation (265,266). Low-dose treatment with the NO donor, FK409, has recently been shown to attenuate small-for-size liver graft injury in rats (267). A relatively high rate of biliary complications ranging from 15% to 64% has also been reported with living-related liver transplantation (268). Nonetheless, availability of right lobe living-related transplantation has been shown to substantially improve the survival rate of adults with FHF, with 50% of patients enrolled in such a program surviving compared to only 6% managed medically while awaiting a cadaveric graft in a recent series from Hong Kong (269).

Rigorous donor selection criteria, both physical and psychological, along with expert postoperative care are required if the safety of the donor is not to be compromised. The reported incidence of donor death in the United States is in the order of 0.2% overall (270), substantially higher than that for kidney donation, which carries a risk of death in the order of 0.03%. Cases of donor death have also been reported from Asia and Europe. Concern has been expressed about the ability to properly assess potential donors on an emergency basis, as is necessary when dealing with FHF. Although in the pediatric setting parents approach the possibility of living-related liver transplantation with enthusiasm, a recent analysis found that

almost two thirds of potential donors were ultimately considered unsuitable for organ donation, with both parents deemed unsuitable in over 20% of cases (271). Living-related transplantation from adult to adult is being increasingly performed in the United States and Europe.

Extracorporeal Liver Support

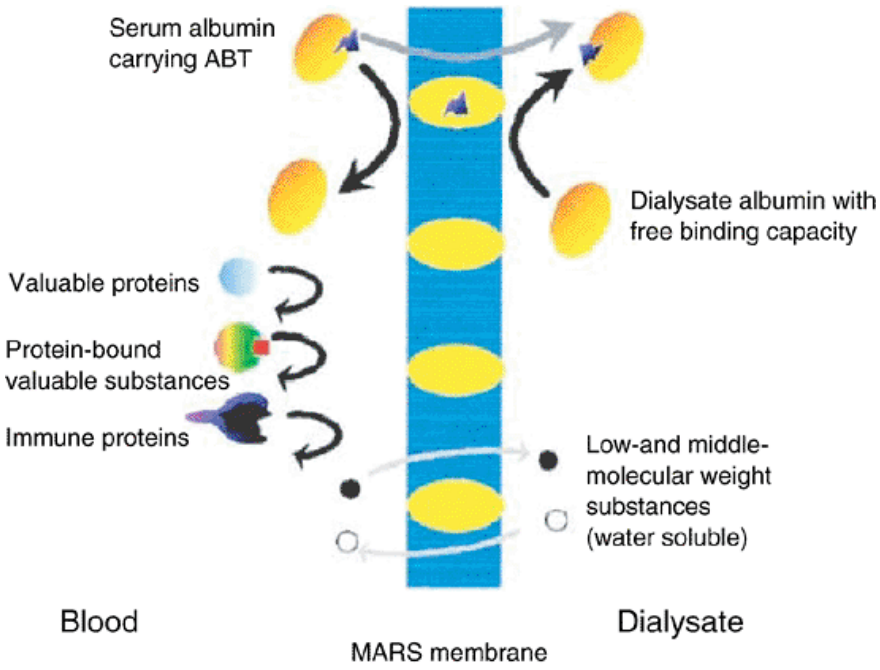
An ever-increasing number of extracorporeal devices of varying complexity are being developed as potential alternatives to auxiliary partial OLT for providing temporary liver support in FHF. Approaches to extracorporeal liver support may be broadly categorized as *artificial*, which contain no biologic component, or *bioartificial*, which include viable liver cells in culture within bioreactors or involve perfusion of the patient's blood through an isolated human or porcine whole liver.

Artificial Liver Support

Early attempts at extracorporeal support based on purely artificial modalities, such as exchange blood transfusion, conventional plasmapheresis, hemodialysis, and hemoperfusion over charcoal or resins (272,273,274,275,276,277,278), were based on the premise that removal of water-soluble and albumin-bound toxins was of paramount importance. The best studied of these artificial modalities is charcoal hemoperfusion. Charcoal is an effective adsorbent for a range of water-soluble molecules in the low-to-middle molecular weight range (up to 5 kDa) and many purported toxins that accumulate in the serum of patients with FHF, such as mercaptans, γ-aminobutyric acid, aromatic amino acids, and fatty acids, but not ammonia (which is heavily ionized at physiologic pH), can be removed or at least reduced in concentration by charcoal hemoperfusion. By contrast, compounds tightly bound to plasma proteins are, in comparison, poorly adsorbed to charcoal (277,278). A number of experimental studies performed in both small and large animal models of FHF reporting a survival benefit with charcoal hemoperfusion, especially with earlier intervention (279,280,281,282), led to initial clinical assessment in patients with FHF and advanced encephalopathy grade (283,284,285). These studies found improved metabolic profiles, including an increased branched-chain amino acid to aromatic amino acid ratio, and at least a transient recovery of consciousness in most treated patients. However, no survival benefit was demonstrated in the only randomized clinical trial performed, even when confounding influences such as etiology of FHF and the jaundice-to-encephalopathy time were taken into account (5). Neither has improved survival in FHF been convincingly demonstrated with any of the other artificial systems listed in the preceding text.

Recent interest has centered on the possible value of large-volume plasmapheresis in FHF, with uncontrolled reports mainly of neurologic improvement and significant reduction in arterial ammonia levels (286,287). Two hemodiadsorption systems that variously combine the processes of hemodialysis and adsorption to charcoal, resins, or albumin, namely the BioLogic-DT device and the molecular adsorbent recirculating system (MARS), are also currently undergoing assessment. As compared to charcoal hemoperfusion alone, removal of tightly protein-bound compounds is enhanced by perfusion over resins or albumin, while reduction in ammonia levels is achieved by dialysis or perfusion over resins (288,289,290,291). The BioLogic-DT system utilizes a flat-bed cellulose membrane for hemodialysis against a suspension of powdered charcoal (with surface area substantially greater than that of granules or beads in a column) and cation exchange resin (292). Nonetheless, clearance of solutes is limited to those that are permeable through the cellulose

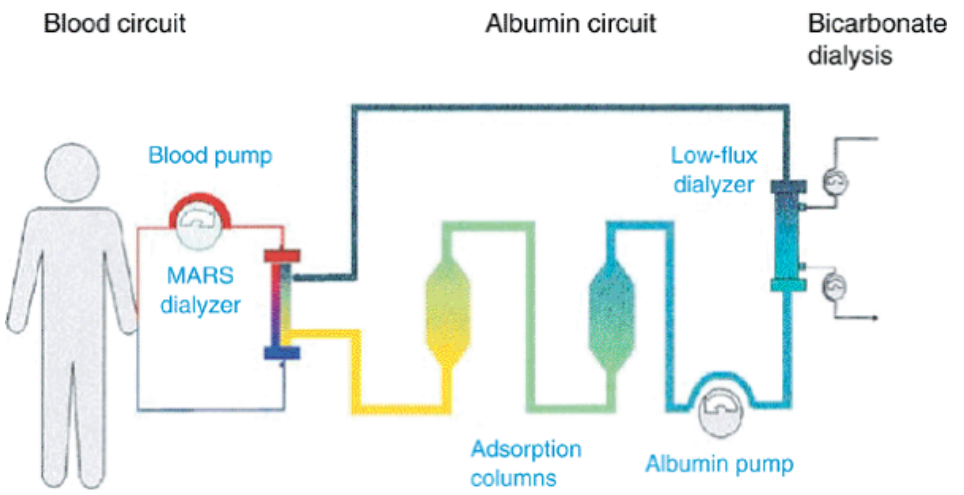
membrane, such that removal of highly protein-bound compounds including unconjugated bilirubin remains limited (293). Preliminary experience in a small number of patients with acute on chronic liver disease has demonstrated instances of improvement in neurologic status after daily, 6-hour treatments (292), although no substantial impact on either encephalopathy grade or overall survival was demonstrated in the setting of FHF (294).



▪ **Figure 21.3** Schematic representation of exchange of albumin-bound toxin (ABT) from serum albumin of the patient through the molecular adsorbent recirculating system (MARS) membrane to the dialysate albumin. (Reproduced with permission from Mitzner SR, Stange J, Klammt S, et al. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. *Liver Transplant* 2000;6:277–286.)

The MARS enables albumin-bound toxins to be removed by dialysis, along with other conventionally dialyzable compounds, as a consequence of the use of a double-sided, albumin-impregnated polysulfone or a hollow-fiber dialysis membrane as a molecular adsorbent in a closed-loop dialysis circuit (295) (Figs. 21.3 and 21.4). Such an approach is based on the long-recognized principle that albumin molecules with free binding sites compete for toxins bound to carrier proteins in perfused blood (284). Incorporation of 5% human albumin in the dialysate results in the transfer of adsorbed toxins in turn from the membrane to the dialysate. The dialysate is then perfused over charcoal and resin adsorbents to remove albumin-bound toxins from the

dialysate albumin and finally dialyzed to remove water-soluble toxins, including ammonia, in readiness for the next cycle. Most experience with MARS to date has been obtained in patients with acute on chronic liver disease. A survival benefit has been suggested in small case series (296) and one controlled study (297). Results of a large, multicenter randomized trial under way in the United Kingdom and Europe are awaited. A recent report documents the successful treatment of fulminant Wilson disease with albumin dialysis, which resulted in the removal of substantial quantities of albumin-bound copper and bilirubin and reversal of multiorgan failure before OLT was performed (298). Other successful experiences have been reported, including spontaneous survival without OLT in cases of acetaminophen overdose and bridging to retransplantation in cases of primary nonfunction of a liver graft (299), although, despite all the difficulties, only with a controlled clinical trial will a definite answer about efficacy be obtained.



▪ **Figure 21.4** Schematic diagram of the molecular adsorbent recirculating system (MARS) circuit. (Reproduced with permission from Mitzner SR, Stange J, Klammt S, et al. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomised, controlled clinical trial. *Liver Transplant* 2000;6:277–286.)

Table 21.7. Various Bioartificial Liver Devices Designed as Alternatives to the Hepatassist and Extracorporeal Liver Assist Device, for which Preliminary Clinical Experience is Available

Name of device	Site of development	Cell type	Additional detoxification components	Comments
TECA Hybrid Artificial Liver Support System	Beijing, China	1–2 × 10^{10} freshly isolated porcine hepatocytes	Charcoal column	Assessed in three patients with FHF; improvement in neurologic status and blood ammonia levels reported (310)
Bioartificial Liver Support System	Pittsburgh, United States	70–120 g freshly isolated porcine hepatocytes mixed with 20% collagen	Nil	Assessed in two patients with FHF; improvement in blood ammonia but not neurologic status

				reported (311)
Radial Flow Bioreactor	Ferrara, Italy	200–230 g freshly isolated porcine hepatocytes	Nil	Assessed in four patients with FHF and three with primary nonfunction; six of seven bridged to OLT; mean reduction in blood ammonia 33% (312)
Hybrid Liver Support System	Berlin, Germany	500–600 g freshly isolated porcine hepatocytes	Nil	Assessed in seven patients with FHF; all bridged to OLT; elevated blood ammonia levels not corrected (313)
Modular Extracorporeal Liver Support	Berlin, Germany	500–600 g freshly isolated human hepatocytes	Albumin dialysis	Assessed in two patients with FHF; both bridged to OLT; improvement in neurologic status in both cases (314)
AMC-Biaortificial Liver	Amsterdam, The Netherlands	1×10^{10} freshly isolated porcine hepatocytes	Nil	Assessed in 12 patients with FHF; 11 of 12 bridged to OLT; 1 patient recovered without OLT; mean reduction in blood ammonia 44% (315)

Bioartificial Hepatic Support System	Udine, Italy	1.5×10^{10} cryopreserved porcine hepatocytes	Nil	Assessed to date in only one patient (with acute on chronic rather than FHF); Neurologic status and blood ammonia levels improved (316)
Hybrid-Bioartificial Liver	Nanjing, China	1×10^{10} freshly isolated porcine hepatocytes	Charcoal column or bilirubin absorption column or plasma exchange	Assessed in 12 patients; clinical data limited—9 patients said to have improved (317)

FHF, fulminant hepatic failure; OLT, orthotopic liver transplantation.

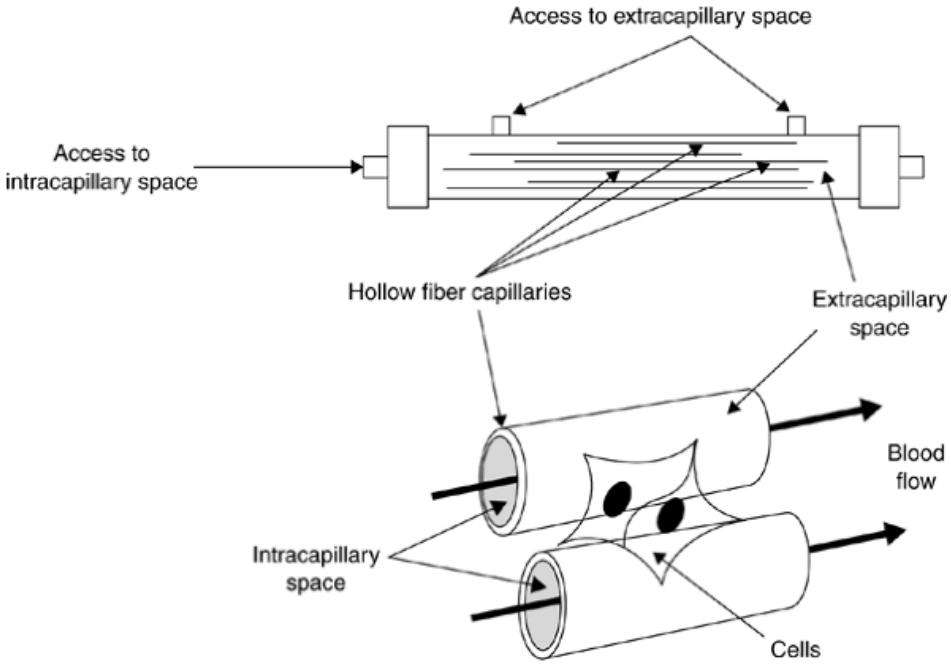
An important consideration in assessing liver support strategies is whether the treatment can reduce the elevated plasma levels of proinflammatory cytokines, which, among other influences, are associated with the development of multiorgan failure and a poor prognosis (300,301). In vitro experiments suggest that perfusion

of plasma over resins or charcoal achieves this aim to varying extents, depending on the adsorbent used and the cytokine in question, whereas hemodialysis using either polysulfone or polyacrylonitrile membranes is ineffective (302). Plasma exchange has been shown to reduce plasma concentrations of TNF-α and IL-6 in patients with FHF and primary nonfunction of hepatic grafts (303,304), although this has not always been the reported experience in other disorders associated with increased circulating cytokine levels (305). Treatment with the BioLogic-DT system results in further elevations in plasma levels of TNF-α and IL-8, possibly as a result of activation of peripheral blood mononuclear cells when passing through the extracorporeal circuit (306). A plasma separation system has recently been incorporated into the circuit, and a preliminary report has indicated a reduction in IL-1β levels with its use (307). An additional, although unproven, concern with some artificial systems is that the treatment may remove hepatotropic factors essential for liver regeneration, such as HGF and TGF-α.

Bioartificial Liver Support

Several bioartificial liver devices, containing viable hepatocytes as a component with the aim of providing additional synthetic and biotransformatory liver functions but differing substantially in both the hepatocyte component and bioreactor design, are currently being assessed either experimentally or clinically (308,309,310,311,312,313,314,315,316,317) (Table 21.7). The simplest design consists of cartridges containing hollow fiber membranes, with perfusion of the patient's blood or plasma through the hollow fiber lumen and culture of the hepatocyte component in the extrafiber space (Fig. 21.5). Although the additional incorporation of nonparenchymal cells may be advantageous for maintaining hepatocyte viability and function in culture as a result of secretion of extracellular matrix (318,319),

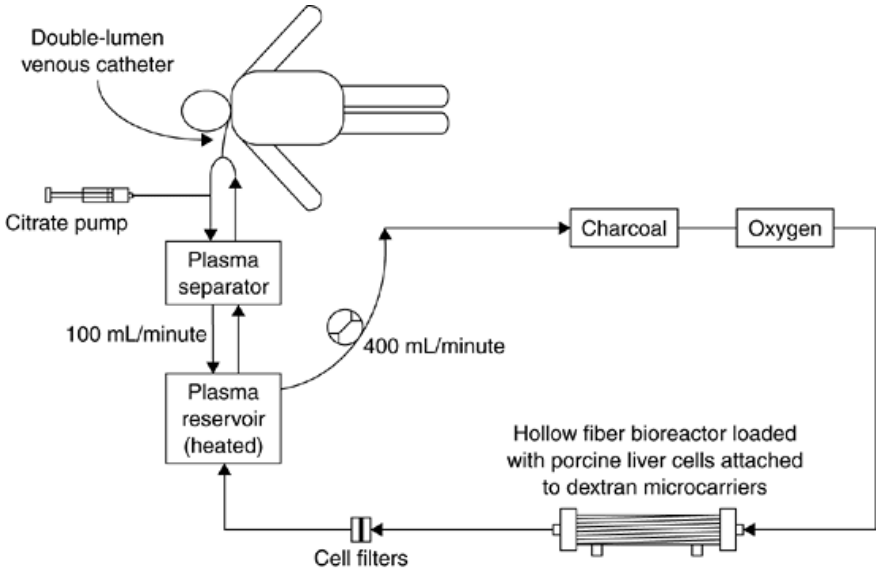
their inclusion might also have deleterious effects if these cells become activated in culture or during clinical application and produce cytokines such as TGF-β associated with the apoptosis of hepatocytes and inhibition of liver regeneration.



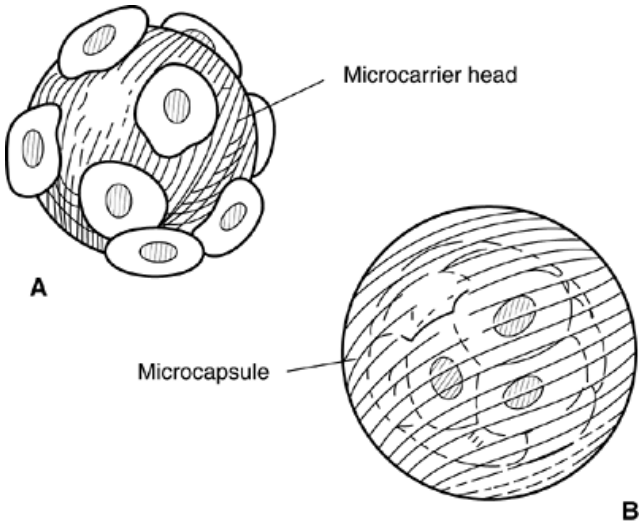
▪ **Figure 21.5** Diagram of a hollow fiber cartridge, on which the bioartificial liver (BAL) and extracorporeal liver assist device (ELAD) are based. The close-up view shows cells attached to the external surface of the hollow fibers. (Reproduced with Permission from: Ellis AJ, Sussman NL, Kelly JH, Williams R. Clinical experience with an extracorporeal liver assist device. In: Lee WM, Williams R, eds. *Acute liver failure*. Cambridge, Cambridge University Press, 1997:255–265.)

Of the devices that have so far undergone preliminary clinical evaluation, most experience is with the "HepatAssist" bioartificial liver support system, which contains porcine hepatocytes as the cellular component, the number approximating 2% of the normal adult hepatocyte mass, and a charcoal column (308) (Fig. 21.6). The hepatocyte component is attached to dextran microcarriers, thereby promoting cell growth and maintenance of cell function in culture (Fig. 21.7). Uncontrolled data suggested more impressive improvement in neurologic rather than metabolic parameters. A prospective, randomized, multicenter controlled trial of the HepatAssist device has recently been completed in the United States and Europe. A significant survival advantage was demonstrated in patients with FHF treated with the device, excluding those with primary graft nonfunction post-transplantation, compared

with controls managed with full medical supportive measures alone (44% reduction in mortality) (308). Although a porcine endogenous retrovirus resistant to lysis by human complement and capable of infecting human cells in vitro has been reported (320), no instances of transmission of this infection on using porcine hepatocytes in the HepatAssist device or others incorporating these cells have yet been documented (321).



▪ **Figure 21.6** Schematic representation of extracorporeal perfusion with the HepatAssist bioartificial liver. (Modified with permission from Rozga J, Podesta L, LePage E, et al. A bioartificial liver to treat severe acute liver failure. *Ann Surg* 1994;218:538–546.)



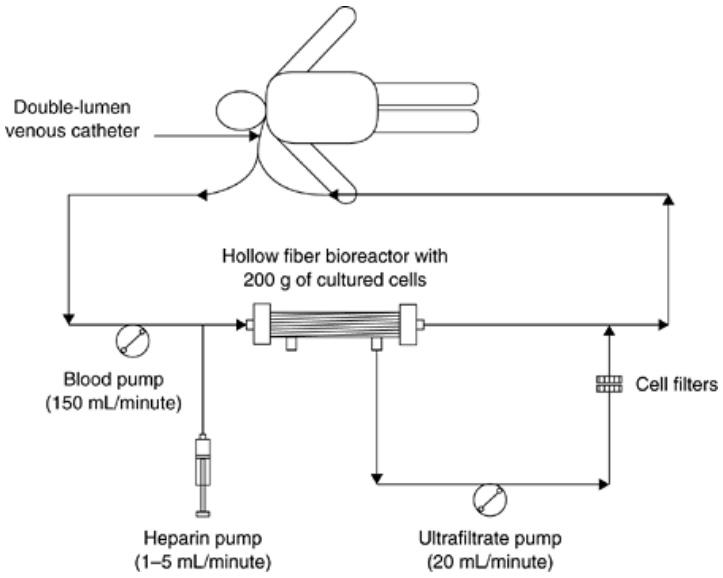
▪ **Figure 21.7** Attachment of hepatocytes to microcarrier beads is a commonly used technique to promote cell growth and maintain differentiated function in culture **(A)**. Encapsulation of cells within semipermeable microcapsules, such as with alginate–polylysine **(B)** is an alternative technique. (Modified with permission from Gerlach JC. Hepatocyte culture and bioreactor design for liver support systems. In: Lee WM, Williams R, eds. *Acute liver failure*. Cambridge, Cambridge University Press, 1997:245–254.)

In the only other controlled experience with a bioartificial liver device so far reported, a pilot but controlled clinical trial of the "extracorporeal liver assist device" (ELAD), which contains a human hepatoblastoma cell line in numbers in the order of 15% of the normal adult hepatocyte mass (309) (Fig. 21.8), demonstrated no statistically significant clinical or metabolic effect in treated patients. Several alternative designs to the HepatAssist and ELAD have been developed and at least preliminarily assessed clinically

(310,311,312,313,314,315,316,317,322). The design characteristics and preliminary clinical experiences with these devices are summarized in Table 21.7. No controlled data are available. Only one device currently undergoing assessment incorporates scarcely available primary human hepatocytes as the cellular component. The functional capacity and suitability or otherwise of immortalized or reversibly immortalized human hepatocytes (323) for use in bioartificial liver support devices represents another area of important investigation.

The use of human, as well as porcine, whole livers for extracorporeal perfusion is also the subject of ongoing interest. Although uncontrolled, metabolic and neurologic improvement was documented in a cohort of 14 patients treated with continuous perfusion until liver transplantation or withdrawal of support. Arterial ammonia levels fell from a median 146 to 83 µmol/L over the first 12 hours, and this reduction persisted for at least 48 hours. Whether pig or human livers were perfused was associated with comparable reductions in

blood ammonia levels. Nine patients were successfully bridged to transplantation (324)



▪ **Figure 21.8** Schematic representation of extracorporeal perfusion with the Extracorporeal Liver Assist Device. (Modified with permission from Gislason GT, Lobdell DD, Kelly JH, Sussman NL. A treatment system for implementing an extracorporeal liver assist device. *Artif Organs* 1994;18:385–389.)

Hepatocyte and Stem Cell Transplantation

The feasibility of isolated hepatocyte transplantation as a therapeutic tool has been demonstrated in studies performed in animals with liver-based metabolic defects, such as analbuminemic and Gunn rats, hypercholesterolemic rabbits, and dogs with impaired purine metabolism (325,326,327,328,329). These studies, in which syngeneic or allogeneic primary hepatocytes in quantities ranging from approximately 2% to 15% of the animal's normal liver cell mass were delivered by intraperitoneal, intrasplenic, or intraportal injection, generally demonstrated satisfactory, if incomplete, correction of the metabolic defect for periods, on occasion, of over 150 days. Approximately 50% to 95% of hepatocytes transplanted in this way are incorporated into liver parenchyma after their deposition in hepatic sinusoids, the remainder sequestrating in other sites, including an estimated 2% to 3% in the pulmonary circulation (330,331,332). The latter are rapidly destroyed in pulmonary capillaries, and the clinical relevance of pulmonary translocation has been considered limited (333). Transient respiratory insufficiency was described in a proportion of treated patients in a recent series (334), while other potential complications include portal vein thrombosis and splenic infarction (335). Successful hepatic engraftment of transplanted hepatocytes is associated with a transient five- to sixfold increase in transaminase levels, along with other evidence of

microcirculatory damage to the host liver (336). The development of portal hypertension precludes the transplantation of larger quantities of hepatocytes, at least as given in one session.

Experience with hepatocyte transplantation in experimental animals with FHF due to chemically induced hepatic necrosis or surgical models of hepatic ischemia or resection has generally demonstrated improved survival, even when small numbers of cells, in the order of only 0.5% to 3% of normal hepatocyte mass, are used (322,327,328,329,330,331,332,333,334,335,336,337,338,339,340). Evidence of enhanced regeneration of the native liver has been reported, possibly as a result of reduction in levels of transforming growth factor β (341). However, transplantation was performed before the induction of FHF in some surgical models, a scenario clearly incongruous to the clinical setting. Furthermore, the relevance of animal models in which the liver is removed to the clinical situation of FHF is uncertain.

Transplantation of freshly isolated or cryopreserved human hepatocytes has, to date, been performed in only a small number of pediatric and adult patients with FHF (342,343,344,345,346,347,348). Instances of mostly short-term improvement in encephalopathy and some metabolic parameters have been recorded both in OLT and

non-OLT candidates, generally after a lag period of several days, possibly reflecting the time necessary for effective engraftment, as in experimental animals (Table 21.8). No randomized, controlled data are currently available. Light microscopic and fluorescent in situ hybridization evidence of persistence of hepatocytes for up to 52 days post-transplantation has been recorded (334). On the basis of experimental evidence that transplanted allogeneic hepatocytes stimulate a weak humoral but strong cell-mediated host immune response (349,350), systemic immunosuppression has been instituted in the clinical applications so far. Alternative strategies for preventing rejection without the need for immunosuppressive drugs, including the transplantation of encapsulated hepatocytes (351), are currently under investigation in animal models.

Table 21.8. Clinical Experiences with Hepatocyte Transplantation in Fulminant Hepatic Failure in Adults and Children

Recipients	N	Transplanted hepatocytes	Assessment of efficacy
Adults			
Bilir et al. (342)	1	1×10^9 cryopreserved human hepatocytes delivered by injection into splenic artery	Improvement in encephalopathy and reduction in prothrombin time; subsequent deterioration precipitated by sepsis
Bilir et al. (343)	6	Cryopreserved human hepatocytes (number not stated) delivered by percutaneous injection	One patient withdrawn because of anaphylactoid reaction; of the remaining five patients, three (60%) survived >72 h (12–52 d), with transient improvement in encephalopathy, blood ammonia levels, and prothrombin times (no patient was considered an OLT candidate)
Bilir et al. (344)	1	3×10^{10} cryopreserved human hepatocytes	No clinical benefit observed and patient died within 18 h.

		delivered through transjugular intraportal injection	
Habibullah et al. (345)	6	6×10^7 ABO-matched fetal hepatocytes per kilogram body weight delivered by intraperitoneal injection	Blood ammonia level reduced in 80% of those in whom serial levels available; survival rate 30% compared with 33% in patients not consenting to hepatocyte transplantation; OLT not available
Strom et al. (346)	2	3×10^7 to 2×10^8 ABO-matched human hepatocytes (depending on availability; ≤0.1% adult hepatocyte mass); cryopreserved hepatocytes used in one case, freshly isolated cells in the other; cells delivered by injection into splenic artery	Each patient with grade III or IV hepatic encephalopathy at time of transplantation; reduction in blood ammonia level in both patients, who underwent OLT after 3 and 10 d; none of three patients for whom no hepatocytes were available for transplantation survived for >3 d
Strom et al. (347)	5	Human hepatocytes delivered by injection into splenic artery as in the preceding entry	Four patients with grade III or IV hepatic encephalopathy at time of transplantation; three bridged to OLT 1–5 d later; one died on day 5 before OLT could be performed; the other patient, with grade I hepatic encephalopathy before hepatocyte transplantation, survived without OLT
Children			
Strom et al. (346,347), Soriano et al. (348)	6	ABO-matched human hepatocytes delivered by injection into splenic artery	Five children with grade IV hepatic encephalopathy at time of hepatocyte transplantation; four of these patients (80%) died within 7 d, despite instances of reduction in serum ammonia levels and requirement for inotropicx support; one of these children recovered without OLT, having received multiple infusions of hepatocytes over a 3-d period; the other child, with grade I hepatic encephalopathy at the time of hepatocyte transplantation, underwent OLT 2 d after the procedure

OLT, orthotopic liver transplantation.

The limited availability of primary human hepatocytes for transplantation represents a major difficulty. Isolation of hepatocytes from livers rejected for liver transplantation is the standard approach, with reported yields in the order of 7 million cells per gram of digested tissue and a mean cell viability of 73% (352). With the source of such hepatocytes in increasingly short supply, the feasibility of harvesting tumor-free hepatocytes from macroscopically normal liver unavoidably removed during hepatic resection for malignancy has recently been demonstrated using an immunomagnetic filtration technique (353). In addition to the potential

new sources of primary human hepatocytes, recent data suggesting that cell viability and function after cryopreservation can be substantially improved with the use of University of Wisconsin solution as a cryopreservation medium (354,355) may enhance the future applicability of hepatocyte transplantation in the FHF setting. Transplantation of hepatocytes with fibrin or other biomatrix, shown in both mice and pigs to result in enhanced hepatocyte engraftment and in maintaining differentiated cell function (356,357), is another technical advance yet to be clinically trialled. The possible benefit to hepatocyte survival of cotransplantation with nonparenchymal cells, as suggested in rats (358), also remains to be established clinically.

In experimental models, repopulation of the whole liver can be obtained with infusion of relatively small numbers of purified hepatic stem cells that are able to replicate at least 100 times without loss of function or malignant transformation, as identified in adult rodent liver (359,360). Repopulation experiments using purified fractions of total liver cell suspensions will be required to identify any such cells in the adult human liver (361). Of note, a bone marrow–derived stem cell capable of repopulating the liver with mature hepatocytes after hepatic injury has recently been described in rodents. In the order of 1.0×10^6 hepatocytes (approximately 0.1% of the total hepatocyte mass) originated from transplanted bone marrow cells by day 13 after liver injury (362). If extrapolated from the animal to the human situation, the time required for engraftment and cell differentiation would be problematic in the acute setting.

On the premise that the fetal liver contains epithelial cells that are in different stages of lineage progression, some of which may exhibit the full regenerative potential of stem cells, transplantation of fetal hepatocytes, rather than the use of adult cells, has been suggested (347). Clinical data so far are limited, and the range of ethical issues pertaining to the use of fetal cells is currently the subject of much debate.

As with approaches to providing extracorporeal support, controlled trials on a multicenter basis in well-defined patient groups and with standardized outcome measures will be essential to properly evaluate the clinical value of hepatocyte transplantation in FHF. A better understanding of mechanisms responsible for the development of liver cell death and complicating multiorgan failure, along with strategies to enhance liver regeneration, may in future enable a more targeted approach to extracorporeal and cell-based therapies for this disorder.

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Chapter 22

Gallstone Disease: Pathogenesis and Treatment

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Key Concepts

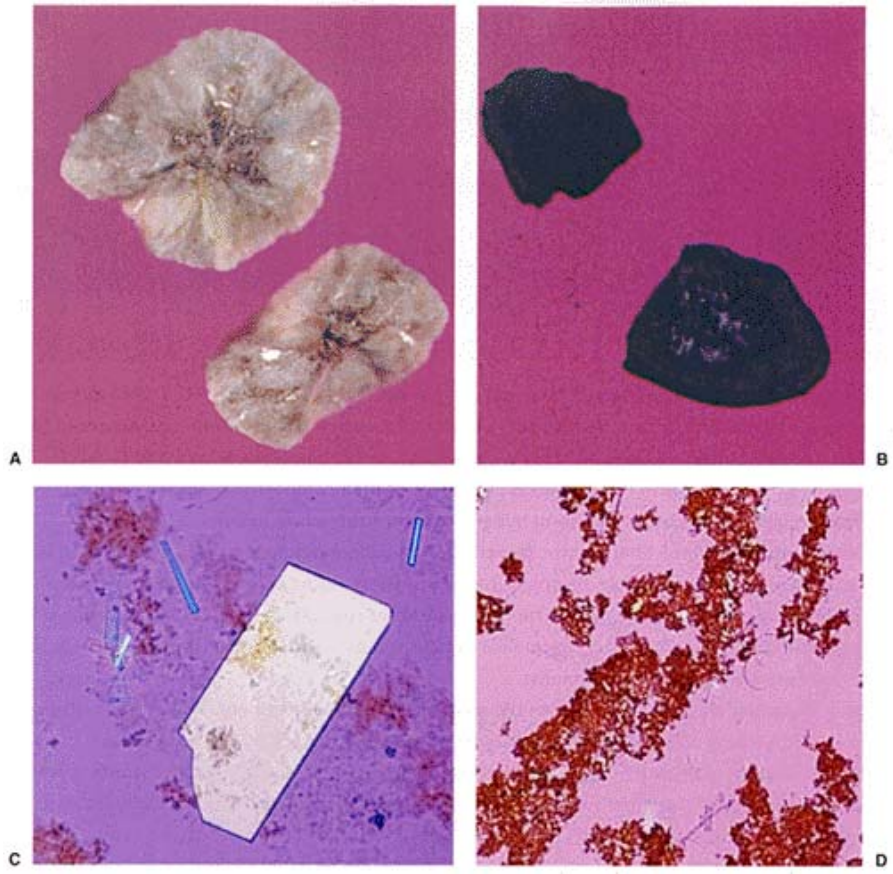
- Gallstones affect approximately 10% of the population in the western world. Common risk factors for stones include age, female sex, obesity, pregnancy, ethnic origin (i.e., American Indian > European > African and Asian), rapid weight loss, chronic hemolytic states, and cirrhosis.
- Gallstones are divided into three types on the basis of their composition. *Cholesterol* gallstone formation involves hepatic cholesterol hypersecretion, gallbladder hypomotility, mucin hypersecretion, and intestinal metabolism of bile salts. *Black pigment* gallstone formation is associated with hypersecretion of conjugated bilirubin, which undergoes conversion to the insoluble unconjugated form, which then precipitates as the calcium salt. *Brown pigment* stones form within the bile ducts as a consequence of biliary stasis and infection, causing degradation and precipitation of biliary components.
- Most gallbladder stones are asymptomatic and do not require treatment. Stones most often present with biliary colic before the development of complications that include acute cholecystitis, biliary obstruction, or pancreatitis. The natural history of bile duct stones is less clear, but because of potentially severe consequences, choledocholithiasis is usually treated, even when asymptomatic.
- Laparoscopic cholecystectomy is the mainstay of treatment of gallbladder stones. Endoscopic therapy is an important tool for common bile duct (CBD) stones, either in addition to surgery or instead of surgery in high-risk patients.

Gallstone disease is one of the most common gastrointestinal afflictions, affecting 10% or more of the population in western countries. Although stones are most often silent, once symptomatic, their management is a major cause of morbidity, with some mortality and major costs to the health care system (1,2,3,4,5,6).

Gallstones are divided into three types on the basis of their composition: Cholesterol, black pigment, and brown pigment. Normally, bile functions as a detergent, facilitating hepatic excretion of otherwise insoluble compounds, as well as intestinal absorption of dietary lipids. Distinct pathophysiologic

mechanisms lead to incomplete solubilization of biliary components such as cholesterol and unconjugated bilirubin (UCB), with formation of either cholesterol or black pigment stones within the gallbladder (Fig. 22.1). Cholesterol stones constitute approximately 80% of stones in patients in western countries and may be composed of almost pure crystalline cholesterol, with minor amounts of UCB, calcium phosphate, mucin, and proteins. The predominant element of black pigment stones is polymerized calcium bilirubinate, with minor quantities of calcium. Their calcium content renders black pigment stones relatively radiopaque on plain radiographs in contrast to cholesterol stones. In addition to the precipitation of these constituents within the gallbladder, pathophysiologic mechanisms in the liver and intestine contribute to cholesterol and black pigment stone formation. In

contrast, brown pigment stones form when bile duct obstruction and bile stasis lead to bacterial infection and degradation of biliary lipids to insoluble compounds, including calcium bilirubinate, along with cholesterol and calcium salts of fatty acids. Epidemiologic risk factors differ for each type of stone and are discussed in the context of their pathophysiology.



▪ **Figure 22.1 A:** Cholesterol stone fractured to show interior structure. Note that individual cholesterol monohydrate crystals can be seen radiating from a pigmented core. **B:** Black pigment stone composed of polymerized calcium bilirubinate. **C:** Microscopic view of cholesterol monohydrate crystals from human bile. **D:** Microscopic view of calcium bilirubinate granules from human bile.

Pathophysiology of Gallstone Formation

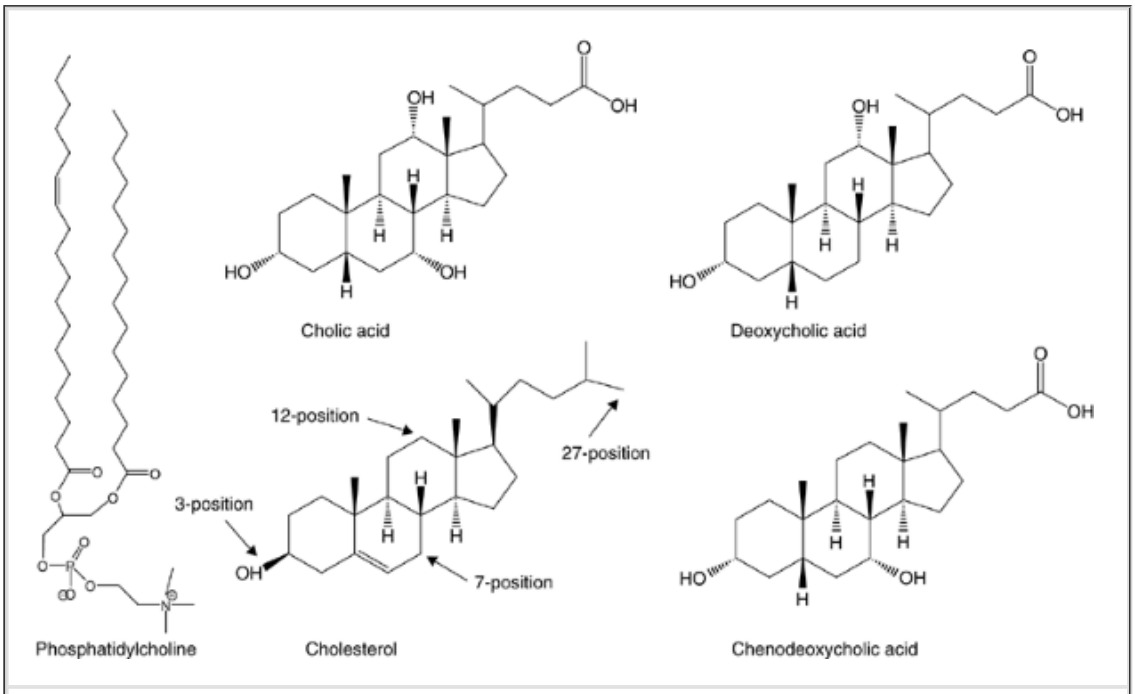
Biliary Lipid Secretion

The principal physiologic functions of bile, absorption of lipids and facilitation of excretion of relatively insoluble compounds, are mediated through the synthesis and secretion of lipid molecules that are either detergents or can cooperatively facilitate solubilization. Major components of bile include bile salts, phosphatidylcholine, and cholesterol, the chemical structures of which are shown in Figure 22.2. (7).

Components of bile

Although bile salts are derived from cholesterol, they are quite water soluble, as compared to the insolubility of cholesterol (approximately 10^{-7} mol/L) (8). The chemical transformation from the parent molecule includes truncation of the hydrocarbon side chain with addition of a carboxylic acid and either two

or three hydroxyl groups. The orientation of the hydroxyl groups allows interaction of the molecule with an air–water or oil–water interface, such that the hydrophilic hydroxyl groups and carboxylic acid can interact with water while the hydrophobic sterol nucleus remains protected from interaction with water. At concentrations above a critical value, denoted by the critical micellar concentration, bile salts self-aggregate to form micelles, with their hydrophobic surfaces on the interior to allow solubilization of otherwise insoluble molecules such as cholesterol (8). Approximately two thirds of secreted bile salts are conjugated with glycine and the other one third with taurine. Conjugation renders bile salts resistant to precipitation at physiologic pH values: Free bile salts precipitate at a pH value less than 7, whereas glycine conjugates precipitate at pH values less than 4.5 and taurine conjugates only below a pH value of 1. Therefore, conjugated bile salts, but not unconjugated bile salts, remain active as detergents throughout the intestine.



▪ **Figure 22.2** Chemical structures of typical biliary lipids. Phosphatidylcholine (lecithin) has varying chain lengths, the most common of which is the 1-palmitoyl, 2-oleyl shown here. Cholic acid and chenodeoxycholic acid are the predominant bile salts synthesized by the liver and are secreted as the glycine or taurine conjugate of the carboxylic acid side chain. Cholesterol is secreted as such and contributes the steroid nucleus for bile salt synthesis. Deoxycholic acid is derived from dehydroxylation of cholic acid by intestinal microflora. Ursodeoxycholic acid differs from chenodeoxycholic acid only in the orientation of the hydroxyl group in the seventh position.

Phosphatidylcholine and cholesterol are insoluble membrane components. Both are amphipathic—phosphatidylcholine with a hydrophilic zwitterionic choline head group and hydrophobic acyl chains and cholesterol with a single hydrophilic hydroxyl group and a hydrophobic sterol nucleus. Bile salts interact with phosphatidylcholine and cholesterol to form spontaneous lipid aggregates, micelles, or vesicles (8). Formation of bile salts into simple micelles enhances the

solubility of cholesterol by approximately 10,000-fold, whereas in the presence of phosphatidylcholine, bile salts form mixed micelles that increase cholesterol solubility by an additional severalfold (9). Exceeding the micellar solubility of cholesterol triggers the formation of unilamellar vesicles composed of a single bilayer of phosphatidylcholine and cholesterol with minor amounts of bile salts (10). Unilamellar vesicles aggregate, fuse, and transform to multilamellar vesicles containing multiple concentric bilayers that can spawn cholesterol crystals (11).

Bile salt synthesis is a highly regulated process that occurs principally in the liver (12,13). Distinct pathways are initiated by hydroxylation at either the 7- or 27-position, mediated by cholesterol 7α-hydroxylase (Cyp7A1) or sterol-27-hydroxylase (Cyp27), respectively (Fig. 22.2). The "classical" pathway of bile acid synthesis is initiated through Cyp7A1 and leads to cholic acid after further hydroxylation at the 12th position. Whereas Cyp7A1 is expressed only in the liver, Cyp27 is expressed widely, including the vascular endothelium. The "alternative" pathway of bile acid synthesis initated by Cyp27 leads to chenodeoxycholic acid, after further 7-hydroxylation by oxysterol-7α-hydroxylase (Cyp7B1) (14). The trihydroxy bile

salt cholate and the dihydroxy bile salt chenodeoxycholate are termed *primary bile salts*. The deoxycholates, ursodeoxycholates, and the monohydroxy lithocholates (with only the 3-hydroxyl group) are the products of intestinal bacterial transformation of primary bile salts: 12-dehydroxylation of cholate, epimerization of the 7-hydroxyl group of chenodeoxycholate, and 7-dehydroxylation of chenodeoxycholate, respectively. The deoxycholates are more powerful detergents and, as discussed later, can play a role in the pathogenesis of cholesterol gallstones (15). Ursodeoxycholate differs from chenodeoxycholate only in the orientation of a single hydroxyl group but does not solubilize cholesterol as well (16). After resorption of more than 95% of bile acids in the ileum by the apical sodium-dependent bile acid transporter (SLC10A2), bile acids are efficiently taken up at the hepatocyte basolateral membrane by the sodium-taurocholate cotransporting polypeptide (NTCP, SLC10A1) (17) and by multiple sodium-independent multispecific organic anion transporters (OATP-A and OATP-C; SLC21A3 and SLC 21A6, respectively) (17). Enterohepatic circulation of bile acids allows efficient conservation of the bile acid pool.

Bile secretion

The sterol nucleus of cholesterol can be excreted only as free cholesterol or after transformation to bile acids. The free cholesterol pool is a major determinant of biliary cholesterol saturation. Cholesterol is stored within the hepatocyte either in intracellular membranes, principally the endoplasmic reticulum, or as cholesteryl esters after esterification by acyl cholesterol acyltransferase-2 (ACAT-2) (18). Cholesterol, free and esterified, enters the hepatocyte through lipoprotein uptake in the form of low-density lipoprotein (LDL), high-density lipoprotein (HDL), or chylomicrons carrying dietary cholesterol. Each process is mediated by apical membrane receptors: The LDL receptor (LDLR) and the LDL-related protein (LRP) receptor for LDL and chylomicrons, and scavenger receptor B type 1 (SR-B1) for HDL. Esterified cholesterol can be stored, hydrolyzed by cholesterol esterases, or exported as newly synthesized very low density lipoprotein (VLDL). Newly synthesized cholesterol can be transformed into bile salts, although the major source of cholesterol for bile salt synthesis is HDL cholesterol, as evidenced by impaired biliary cholesterol excretion in mice with decreased levels of SR-B1

(19).

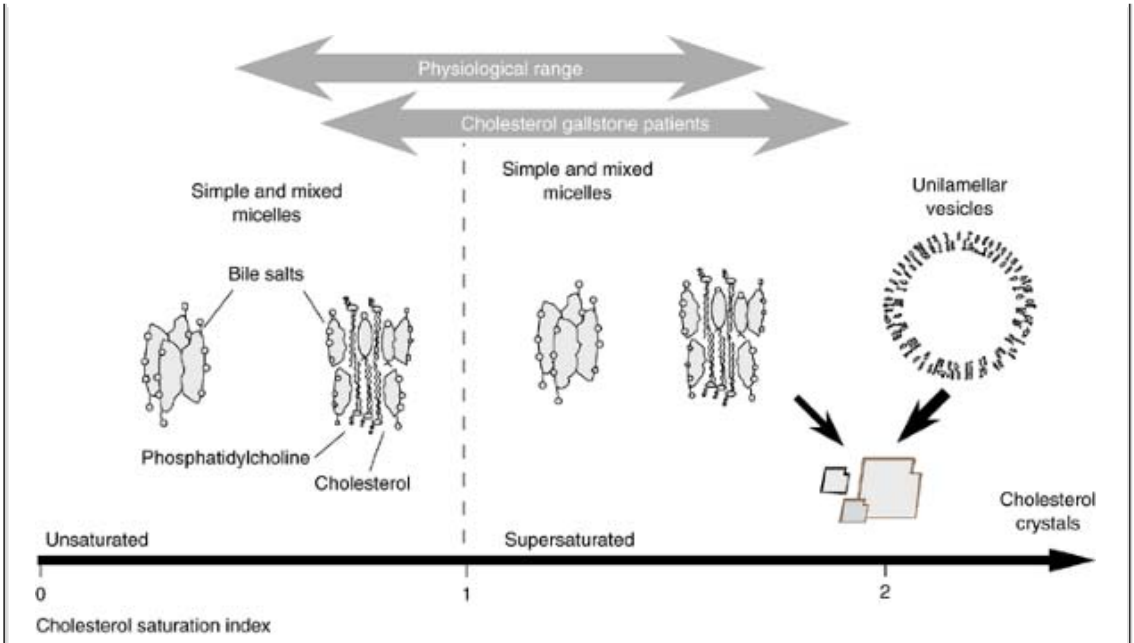
Biliary lipid secretion occurs through the coordinated expression of multiple transporters at the canalicular membrane (17,20). Unconjugated bile salt secretion occurs predominantly through the bile salt export protein (Bsep, ABCB11), a member of the adenosine triphosphate–binding cassette superfamily (21), whereas glucuronidated and sulfated bile salts are excreted by the canalicular multispecific organic anion transporter multidrug resistance protein (MRP2) (22). Phosphatidylcholine is transported from the inner to the outer canalicular membrane by a transmembrane transferase, or flippase, encoded by the murine gene product mdr2 and the human gene product MDR3 (ABCB4), the absence of which is associated with almost total ablation of phosphatidylcholine and cholesterol secretion in bile (23). Cholesterol transport at the canalicular membrane is mediated by two gene products (ABCG5 and ABCG8), which form a heterodimer transport protein (24). However, residual cholesterol excretion is observed in patients with sitosterolemia, in whom both gene products are defective (25); this suggests that cholesterol excretion into bile may occur by alternative mechanisms such as spontaneous transmembrane movement ("flip-flop") (26). Multiple monogenetic defects in canalicular transporter genes, as well as those of the enzymes of bile acid biosynthesis, have been identified with phenotypes ranging from cholesterol gallstone disease to severe early childhood cholestasis (27).

Once in the canalicular lumen, bile salts initiate vesiculation of phosphatidylcholine and cholesterol, culminating in the release of unilamellar vesicles (28). Despite the presence of other phospholipids in the canalicular membrane, phosphatidylcholine is virtually the sole phospholipid secreted in bile, consistent with the higher affinity of bile salts for phosphatidylcholine as compared with sphingomyelin (29). The mechanism for the selectivity for phosphatidylcholine versus sphingomyelin is likely to be physicochemical in part, given the greater susceptibility of phosphatidylcholine to bile salt solubilization (29,30). As bile is concentrated, bile salt concentration increases above the critical micellar concentration and solubilizes phosphatidylcholine and cholesterol as mixed micelles (31). The cholesterol content of bile in part depends on the detergent capability of the bile salt species present. More highly detergent hydrophobic bile salts more effectively extract cholesterol from the canalicular membrane to produce bile with relatively higher cholesterol content (32).

The relative cholesterol content of bile is most often expressed as the cholesterol saturation index (CSI), which is the amount of cholesterol present divided by the maximum micellar solubility of cholesterol by the bile salts and phosphatidylcholine present. This can be expressed on a ternary phase diagram showing the relative quantities of bile salts, phosphatidylcholine, and cholesterol, where the apices of the triangle represent systems with 100% of each component in aqueous solution or suspension (9).

The concentration of cholesterol in human bile fluctuates around the limit of cholesterol solubilization (Fig. 22.3). At lower bile salt secretion rates, such as during fasting when less bile salts are being returned in

the portal circulation, the relative cholesterol content increases (33). Patients with gallstones have somewhat higher CSI values; however, there is great overlap in the distribution of CSI values in bile between healthy individuals and patients with cholesterol gallstones (34).



▪ **Figure 22.3** Cholesterol saturation in bile typically fluctuates around a value of unity for the cholesterol saturation index (CSI). Below a CSI of 1, cholesterol is solubilized in simple and mixed micelles, whereas above a CSI of 1, unilamellar and, ultimately, multilamellar vesicles are formed, from which cholesterol crystallization can occur. (Adapted from Donovan JM. Pathogenesis of gallstones. In: Feldman M, LaRusso NF, eds. *Gastroenterology and hepatology: The comprehensive visual reference, vol 6, gallbladder and bile ducts*. St. Louis: Mosby, 1997:7.1–7.22.)

Bile acid and cholesterol homeostasis is tightly regulated. The liver X receptor (LXR), which forms heterodimers with the retinoid X receptor (RXR), appears to be the major mediator of cholesterol homeostasis (35). The major ligands of LXR are oxysterols; activation by elevated levels of intracellular cholesterol stores triggers coordinated changes that serve to decrease body cholesterol stores. Among the target enzymes whose activity is enhanced are Cyp7A1 effecting conversion of cholesterol to bile acids, ABCG5/ABCG8 effecting biliary cholesterol excretion, and ABCA1 serving to decrease intestinal cholesterol absorption (36) while downregulating the ileal bile acid transporter to enhance bile acid excretion (35). Similarly, the farnesoid X receptor (FXR), which also forms RXR heterodimers, acts as the major mediator of bile salt homeostasis. The major ligands of FXR are the hydrophobic bile acids, particularly chenodeoxycholate. Elevated intrahepatocyte bile acid levels trigger several homeostatic mechanisms: Cyp7A1 is downregulated, decreasing bile acid synthesis; whereas ABCB4 and ABCB11 are upregulated, increasing phosphatidylcholine and bile acid excretion at the canalicular membrane. The critical importance of FXR is illustrated by the observations that FXR-null mice display a phenotype of cholesterol-supersaturated bile, whereas treatment with an FXR agonist prevents the development of cholesterol gallstones (37).

Cholesterol Gallstones

Overview of factors in cholesterol gallstone pathogenesis

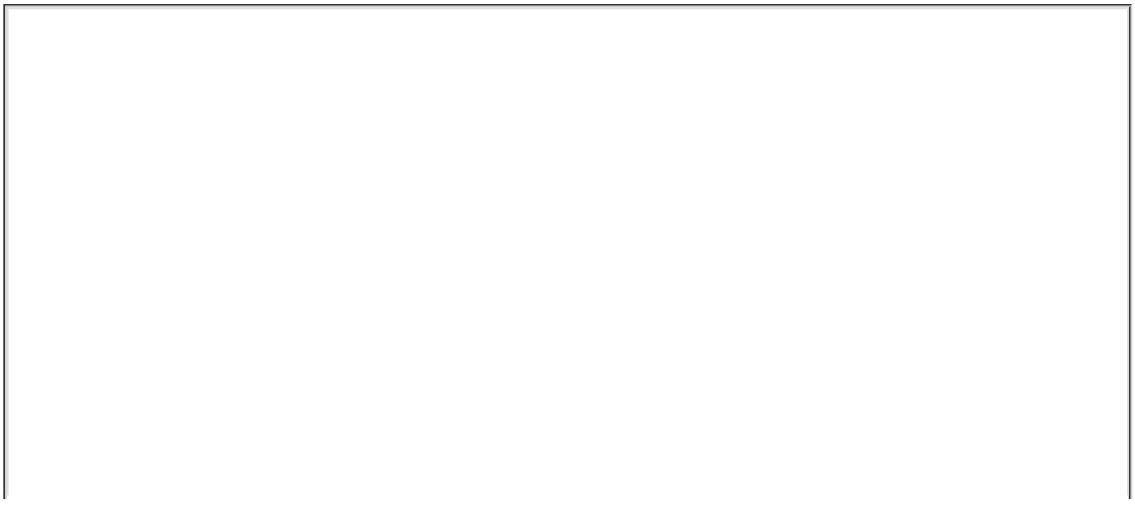
Although cholesterol supersaturation is absolutely required for cholesterol stone formation, multiple factors interact during the formation of stones. As discussed later, cholesterol supersaturation of bile also depresses gallbladder motility. Consequently, the impaired storage capacity of the gallbladder allows a larger fraction of newly secreted bile to be directed to the intestine, where bile salts undergo bacterial transformation to more hydrophobic bile salts such as deoxycholate and lithocholates. Enterohepatic recycling of deoxycholic acid and more efficient extraction of cholesterol at the canalicular membrane can lead to more cholesterol-supersaturated bile. Moreover, cholesterol-supersaturated bile triggers mucin hypersecretion, which accelerates cholesterol crystal

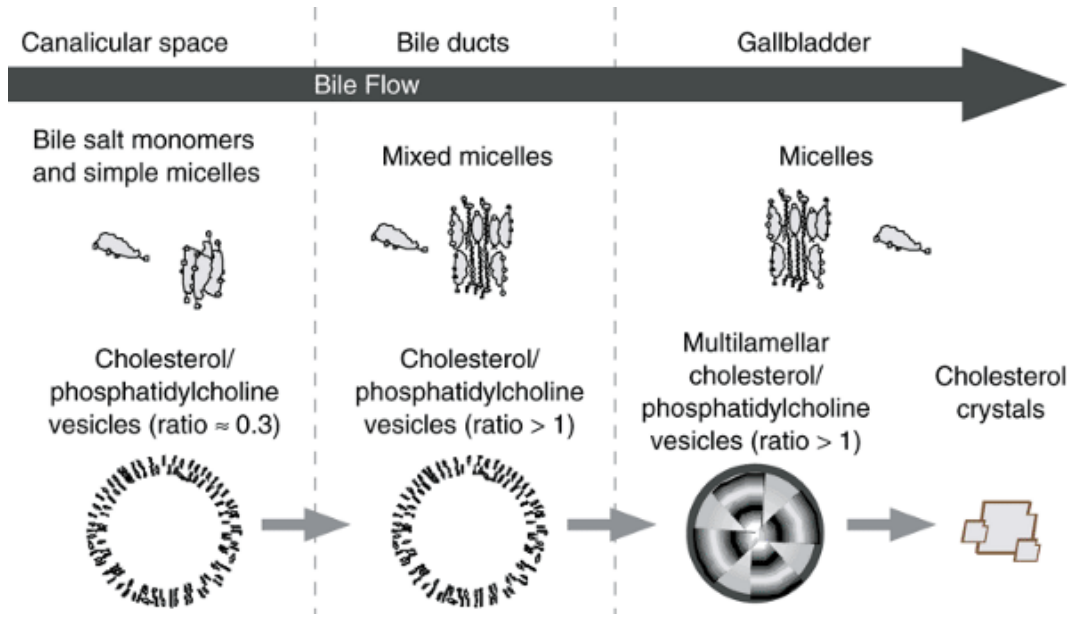
formation and forms a mucous gel layer that impairs biliary clearance of cholesterol crystals and incipient stones (38). Additional factors, such as pro- and antinucleating substances and possibly bacteria, are also thought to play a role in the processes leading to cholesterol crystallization and gallstone formation.

Cholesterol crystallization

When bile is supersaturated with cholesterol, dissolution of vesicles to micelles is incomplete (Fig. 22.4). The remaining vesicles become highly enriched with cholesterol, reaching cholesterol to phosphatidylcholine ratios of 2 or more, which are thermodynamically unstable and cause fusion to produce multilamellar vesicles (10). The similarity of the molecular arrangement of cholesterol in bilayers with that of the crystal structure of cholesterol monohydrate suggests that multilamellar vesicles are crucial in spawning cholesterol crystal formation, as has been observed microscopically (39).

Cholesterol exists in gallstones as the thermodynamically favored cholesterol monohydrate form (40). These are rhomboid, platelike microcrystals that aggregate to form the macroscopic stones (41). Under conditions when the driving force for cholesterol nucleation is high, initial crystallization can also occur in other crystal forms, including cholesterol filaments, helices, and ribbons (42). Some of the earliest crystal forms are intermediate polymorphs of cholesterol monohydrate, with a shift in the water molecules in relation to the cholesterol (43,44). As crystallization proceeds, these structures disappear in favor of the more stable cholesterol monohydrate crystals (45).





▪ **Figure 22.4** Cholesterol is secreted in the form of cholesterol/phosphatidylcholine vesicles that are unsaturated with cholesterol in the proximal biliary tree. As bile becomes more concentrated distally, bile salt concentrations rise, causing preferential extraction of phosphatidylcholine from unilamellar vesicles. Consequently, cholesterol-enriched vesicles are formed that are thermodynamically unstable. In the gallbladder, vesicles aggregate and fuse and ultimately spawn crystalline cholesterol. (Adapted from Donovan JM. Physical and metabolic factors in gallstone pathogenesis. *Gastroenterol Clin North Am* 1999;28:75–98.)

The rate of cholesterol crystal formation is higher in bile from patients with cholesterol gallstones than in bile from healthy controls (46). When determined in vitro, cholesterol crystals form within a few days when bile from patients with cholesterol gallstones are incubated at 37°C but may not form in several weeks in bile from control patients. Numerous pronucleating and antinucleating proteins have been identified (47). Proteins that promote nucleation include mucin, fibronectin, α_1-glycoprotein, *N*-aminopeptidase, haptoglobin, and immunoglobulins, whereas antinucleating proteins include apolipoproteins A-I and A-II and certain immunoglobulins. The consensus view is that differences in concentrations of these proteins do not distinguish a subgroup of patients destined to develop gallstones (48). The exception may be the pronucleating agent mucin, which plays a crucial role in inhibiting cholesterol crystal clearance from the gallbladder and in internally scaffolding mature cholesterol gallstones (49). Additionally, the process of cholesterol crystal formation can be accelerated by increased cholesterol content, total lipid concentration, and relative bile salt content and hydrophobicity (1).

Role of the gallbladder

Gallbladder motor function and mucosal function play critical roles in the formation of gallstones (38,50). Gallbladder filling is a dynamic process characterized by interdigestive partial contractions allowing significant turnover

of bile during its residence in the gallbladder (51). Impaired gallbladder contractility in patients with cholesterol gallstones prevents complete clearance of nascent crystals, presumably facilitating their

growth to mature gallstones (52). The increased cholesterol content of bile alters the cholesterol content of the sarcolemmal membrane and impairs contractility (53).

Hypersecretion of mucin, a heavily glycosylated protein that binds hydrophobic biliary lipids and accelerates cholesterol crystal formation (49), is a central event in both cholesterol and black pigment gallstone formation that can be inhibited by prostaglandin inhibitors (54). Further, this highly viscous gel entraps cholesterol crystals and is a component of biliary sludge (55). The biochemical composition (i.e., lipids, proteins, and bilirubin) of bile is also modified during residence in the gallbladder, contributing to sludge formation (50).

The process by which cholesterol gallstones grow is not well characterized but presumably involves both accretion of cholesterol monohydrate crystals and, to a lesser degree, the enlargement of existing crystals (56). Cross-sections of cholesterol stones show a radial arrangement of cholesterol monohydrate crystals, cemented together by a mucin matrix. Frequently, cholesterol stones have pigmented rings, containing small amounts of calcium bilirubinate (57), possibly caused by fluctuations in bile composition during stone formation. In general, stones from a single gallbladder are of a single type (cholesterol or black pigment), with similar compositions. However, a single gallbladder can contain a single large stone or dozens of tiny stones, possibly through different mechanisms (58).

Role of intestinal transit

Bacteria may influence gallstone formation not only directly in bile but also indirectly within the intestines (59). Intestinal bacteria can increase deoxycholate production in the gut. Elevated levels of deoxycholate are associated with cholesterol gallstone formation (60). This hydrophobic bile acid has been shown to increase biliary cholesterol secretion, and thereby cholesterol saturation and bile lithogenicity. The observation that cholesterol gallstones are prevalent in patients with acromegaly treated with octreotide led to a series of studies demonstrating that these patients have impaired gallbladder contractility and prolonged intestinal transit times, with increased intestinal production of deoxycholate and increased relative amounts of biliary deoxycholate and cholesterol (61,62). The central mediator appears to be increased conversion of the primary bile salt cholate to the secondary bile salt deoxycholate. Enhanced enterohepatic circulation of deoxycholate leads to cholesterol hypersecretion because deoxycholate is a more potent detergent at the canalicular membrane. Additionally, increased biliary deoxycholate levels accelerate cholesterol crystal formation (63). Further, interventions that increase intestinal transit time also trigger cholesterol stone formation in animal models, a process that can be prevented by agents that enhance intestinal transit (64). Although not all patients with cholesterol gallstones have elevations in biliary deoxycholate levels (65), it is likely that prolonged intestinal transit time and increased deoxycholate levels together are contributing factors in a subgroup of patients without other obvious risk factors (66). Patients with gallstones have been shown to harbor an increased mass of bacteria (mainly *Clostridium* spp) capable of 7α-dehydroxylation and deoxycholate production (61). Moreover, antibacterial

treatment has been shown to decrease biliary deoxycholate concentration and bile lithogenicity (67)

Genetics

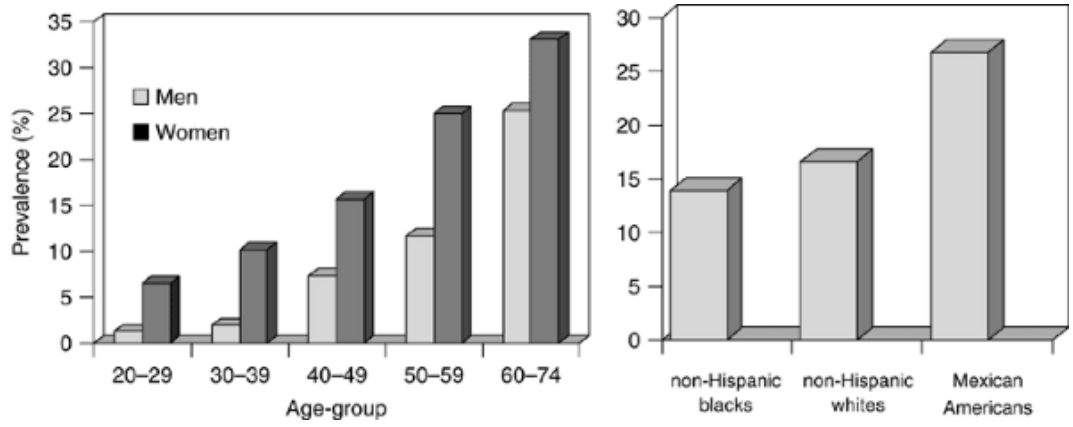
A genetic component of gallstones was initially suggested by a host of epidemiologic data (68). In particular, the strikingly high prevalence of gallstones in specific populations such as American Indians strongly supports a genetic background of gallstone disease (69). This clustering of gallstones does not necessarily prove the genetic basis of the disease but could also reflect environmental factors. However, additional data from family studies with spouses, as well as mono- and dizygotic twins, have provided more firm evidence that gallstone formation is indeed genetically determined (70,71). In this background, a series of studies in the mouse model have looked for underlying genetic defects leading to gallstone formation. By using the genetic tool of Quantitative Trait Locus (QTL) analysis with inbred mice, a number of gallstone susceptibility genes, have been identified, which they have termed *Lith genes* (72,73). To date 16 Lith gene candidates have been identified on 12 different mouse chromosomes, among which several seem to be of greatest potential relevance to cholesterol gallstone formation: Lith 1 on chromosome 2, Lith 2 on chromosome 19, and Lith 9 on chromosome 17. Likely candidate genes include ABCB11 (encoding for the bile salt export pump), ABCC1 (an organic anion transporter), ABCG5/G8 (the cholesterol transporter), and FXR (modulating bile acid metabolism), respectively (74). Absolute confirmation of the relevance of these findings, however, requires verification by experiments with knockout and knock-in animals (75).

Epidemiologic studies in humans have revealed several genetic associations with gallstone disease. The ApoE4 genotype has been shown to predispose to gallstone formation in some studies, but not in others (76,77). Patients with progressive familial intrahepatic cholestasis (PFIC) type 3 have defects in *ABCB4*, which encodes for the phophatidylcholine flippase (ABCB4) (78). Consequent decreases in the phosphatidylcholine to cholesterol ratio in bile result

in decreased cholesterol solubility and accelerated cholesterol crystallization (79). Patients with benign recurrent intrahepatic cholestasis (BRIC) type 2 have mutations in ABCB11, the main canalicular bile salt transporter (80). However, the phenotype of mice strains susceptible to cholesterol gallstone formation has been reported to be that of increased activity of the bile acid transporter with cholesterol gallstone formation (81); hence, it is unclear in humans whether this mutation is associated with gain or loss of function. Patients having defects in the rate limiting enzyme of bile acid synthesis (CYP7A1) have hypercholesterolemia and gallstones and evidence of disproportionate bile acid synthesis through the alternative pathway (82). Observed increases in hepatic cholesterol content and the chenodeoxycholic acid to cholic acid ratio could lead to cholesterol-supersaturated bile, the former by enhancing cholesterol secretion and the latter by augmenting cholesterol solubilization from the canalicular membrane by the more hydrophobic bile acid chenodeoxycholate. Polymorphisms in the promoter region of the gene for the cholecystokinin (CCK) receptor have been identified in patients with gallstones (83). Presumably, impaired gallbladder contractility could increase the risk for gallstones. In general, genetic mutations associated with gallstones tend to present as cholelithasis at a young age.

Bacteria and gallstones

Although traditionally bacteria were not believed to be involved in the pathogenesis of cholesterol gallstones as opposed to brown pigment stones, this concept has been challenged in recent years. Using molecular biology techniques, bacterial deoxyribonucleic acid (DNA) can be found in most cholesterol gallstones (84). Bacteria may form a biofilm that serves as a matrix for crystals and stones (85). They can influence bile lithogenicity by phospholipase activity or the production of mucin, prostaglandins, or oxysterols (86). Recently, several *Helicobacter* species have been found in the intestines, bile, and even gallstones of laboratory animals and humans (87,88). Moreover, pathogen-free C57L mice failed to develop gallstones on a lithogenic diet (88) Although mounting data suggest that gallstone formation may be significantly influenced by bacteria and bacterial products, the exact role of bacteria in cholesterol gallstone pathogenesis still remains to be determined.



▪ **Figure 22.5** Prevalence of gallstones in the US population as determined by ultrasonography. With increasing age, gallstone prevalence increases, and in each age-group, women more commonly have gallstones than men. Mexican Americans have a higher prevalence than non-Hispanic whites or non-Hispanic African Americans. (Data from Everhart JE, Khare M, Hill M, et al. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999;117:632–639.)

Epidemiology and clinical risk factors

Clinical risk factors for cholesterol gallstones are most often associated with biliary hypersecretion of cholesterol. Large epidemiologic surveys performed in countries where cholesterol stones are most prevalent have demonstrated that gallstones are common and most frequent in women and occur with increasing frequency at older ages (89,90). Results are similar in studies using abdominal ultrasonography as a screening tool to identify both asymptomatic and symptomatic stones and in studies examining the prevalence of previously diagnosed gallstone disease. The prevalence of gallstones increases with age (Fig. 22.5), apparently as a result of a decrease in bile salt synthesis, leading to an increase in biliary cholesterol saturation (91).

The propensity for the development of gallstones varies widely among populations (92). The highest prevalence is found among populations of American Indian ancestry, such as the Pima Indians, but extending to North and South American populations with significant genetic contributions from American Indian populations (93). Northern Europeans have a higher prevalence of stones than southern Europeans (5), whereas gallstones are less common among populations of Asian and African ancestry (90). Ancestral

exposure to adverse climates with selection for efficiency of nutrient use has been suggested as a common factor (94). An increased risk occurs within kindreds but does not appear to be mediated by a single gene in most patients.

Both endogenous and exogenous estrogens are associated with increases in gallstone prevalence. At every age, women are more likely than men to harbor stones, with the greatest excess prevalence occurring in the premenopausal years when estrogen levels are highest in women (90). Oral contraceptives (95) and estrogen replacement therapy (96) are associated with modest increases (relative risk <1.8) in gallstone prevalence; because of the much lower prevalence of gallstones in younger women, the effect is of more potential consequence for postmenopausal women. Pharmacologic estrogen administration accelerates stone formation in men as well (97). The common mechanism appears to be upregulation of the LDL receptor (98), with consequent increases in the biliary cholesterol pool and biliary cholesterol saturation (99). In the Pima Indians, puberty in girls but not in boys is accompanied by an increase in biliary cholesterol saturation (100).

Pregnancy is a particularly high-risk period for gallstone formation (101,102). In addition to the association of elevated estrogen levels with cholesterol hypersecretion, elevated progesterone levels impair gallbladder contractility (103). The prevalence of biliary sludge rises throughout pregnancy, reaching more than 30% at delivery (101,102). Although in most women sludge disappears over the postpartum period, stones form in up to 10%.

Obesity and weight loss appear to be independently associated with gallstone disease (104,105). The activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is increased in obesity. The increased synthesis of cholesterol enriches the hepatic pool and hence canalicular cholesterol secretion (106). During rapid weight loss, gallbladder contraction is impaired and bile cholesterol saturation increases (107), leading to high prevalence rates of cholesterol gallstones in patients after extremely hypocaloric diets or in those who have undergone antiobesity surgery (108,109).

Dietary factors associated with gallstone disease include a modest negative correlation with vegetable protein intake (110) and a positive correlation with consumption of legumes (111) and trans-fatty acids (112). Increased physical activity is associated with decreases in clinical gallstone disease (113). Increase in carbohydrate intake seem to be associated with gallstones (114)

Hypertriglyceridemia has been associated with biliary hypersecretion and gallstone disease (115), apparently mediated through the effect of bile salt malabsorption (116) with decreased activation of FXR-dependent pathways that decrease serum triglycerides (117). Decreased activation of FXR in animal models is associated with cholesterol supersaturation (37). Plasma cholesterol levels have been negatively correlated with gallstone disease, whereas reduced HDL cholesterol levels have been associated with an increased risk (118), consistent

with the role of HDL in supplying biliary cholesterol. Fibric acid derivatives and, to a lesser extent, nicotinic acid increase biliary cholesterol saturation as a consequence of decreasing serum cholesterol level. In contrast, the effect of HMG-CoA reductase inhibitors on decreasing hepatic cholesterol has been associated with decreased biliary cholesterol saturation and even dissolution of gallstones in animal models (119). Apolipoprotein E-4 genotype has been associated with increased biliary cholesterol saturation, cholesterol stones, and rapid recurrence after lithotripsy treatment, leaving the gallbladder in situ (76), although not in all populations (77).

Gallstones have been associated with diabetes mellitus and insulin resistance (120,121). A large part of this association reflects the relation of obesity to type 2 diabetes, but there is an independent effect of diabetes, likely mediated through impaired gallbladder emptying (122) or a direct effect of insulin to increase biliary cholesterol saturation (123). Of note, there are strong correlations among risk factors for gallstone disease associated with syndrome X such as obesity, insulin resistance (120), and hypertriglyceridemia, as well as negative associations with physical activity (124). Both high carbohydrate intake and diets with high glycemic indices are also associated with an increased risk of gallstone formation (114,125). The recent appreciation that FXR has effects on glucose metabolism suggests that the association between diabetes and gallstone formation is based on metabolic cross-talk between lipid and glucose homeostasis rather than common epidemiologic risk factors (126)

Patients who have spinal cord injuries have a high prevalence of gallstone disease, which may present atypically because of impaired sensory innervation (127).

Intestinal hypomotility has been suggested to be a major etiologic factor in a subset of patients with gallstones (128). As discussed in the preceding text, patients with acromegaly treated with the somatostatin analog octreotide are at substantial risk for the development of cholesterol gallstones (129).

Black Pigment Gallstones

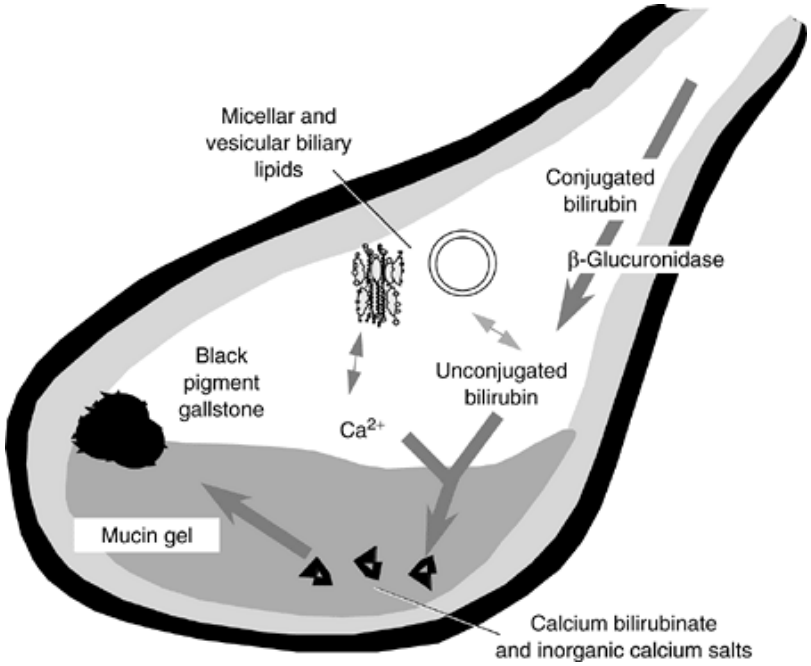
Black pigment gallstones predominantly contain the calcium salt of UCB, with minor amounts of calcium phosphate in a mucin matrix (130). Conjugated bilirubin, the degradation product of heme, is the predominant form secreted in bile after its synthesis in the hepatocyte by conjugating two glucuronide residues

with the tetrapyrrole rings of UCB (131,132). Small amounts of nonenzymatic degradation (133), along with tissue-derived β-glucuronidase, transform conjugated bilirubin to UCB. Bile salt micelles can solubilize minor amounts of UCB (131), but in the presence of calcium, UCB precipitates as the calcium salt (Fig. 22.6). After precipitation, calcium bilirubinate undergoes a free-radical–induced polymerization to an insoluble polymer (134,135). As is the case for cholesterol stones, mucin glycoproteins also form a structural matrix of black pigment stones.

The major determinant of the amount of conjugated bilirubin secreted is the requirement for degradation of heme to bilirubin for excretion. Therefore, conditions in which heme degradation is increased predispose to black pigment gallstone formation (1). An increased prevalence is observed in chronic hemolytic anemias such as sickle cell anemia and β-thalassemia. Patients with chronic liver disease have a substantially increased risk of gallstones (136), most of which are

of the black pigment type. In addition to increased hemolysis in the presence of portal hypertension and splenomegaly, in cirrhosis, secretion of bile salts is diminished, reducing the capacity of bile to solubilize UCB in bile salt micelles (137).

Although patients with Crohn's disease have been observed to have a twofold to threefold increase in the prevalence of gallstones compared with the general population (138), the mechanism has only recently been elucidated (139). Contrary to previous belief that bile acid malabsorption could contribute to biliary cholesterol supersaturation, patients with Crohn's disease have decreased biliary cholesterol saturations (140). Under normal conditions, conjugated bilirubin undergoes bacterial deconjugation in the colon, with further metabolism of bilirubin to urobilinogens. However, in Crohn's disease, ileal disease can lead to impaired bile salt reabsorption and spillover of bile salts into the colon, where they mediate absorption of UCB (139) This enhanced enterohepatic circulation produces an increase in hepatic conjugated bilirubin secretion, which initiates the cascade leading to UCB precipitation and black pigment stone formation.



▪ **Figure 22.6** Formation of black pigment gallstones. A relative increase in conjugated bilirubin secretion results in an increase in unconjugated bilirubin level. Deconjugation occurs by tissue β-glucuronidases or nonenzymatically. Biliary lipid aggregates can only partially solubilize calcium bilirubinate and precipitation ensues, along with inorganic (carbonate and phosphate) calcium salts. As for cholesterol stones, mucin provides a scaffolding for the formation of mature black pigment gallstones. (Adapted from Donovan JM. Physical and metabolic factors in gallstone pathogenesis. *Gastroenterol Clin North Am* 1999;28:75–98.)

Patients on total parenteral nutrition (TPN) have gallbladder stasis secondary to decreased stimulation of CCK release. Additionally, intestinal hypomotility may allow increased resorption of UCB, contributing to increases in biliary secretion of

conjugated bilirubin, as has been observed in animal models (141). These factors contribute to the nearly universal presence of biliary sludge and frequent development of black pigment gallstones observed in patients receiving TPN (142).

Brown Pigment Gallstones

The pathogenesis of brown pigment stones appears to differ in various parts of the world. In general, brown pigment stones form when bile duct obstruction and

bile stasis lead to bacterial infection and degradation of biliary lipids to insoluble compounds including calcium bilirubinate, along with cholesterol and calcium salts of fatty acids (85,143). In countries with a western, high-fat diet, brown pigment stones most often form in the common bile duct (CBD) after cholecystectomy. The initiating event is the presence of a foreign body, typically a retained stone and occasionally suture material. Transient bacterial colonization in the presence of a foreign body can trigger the formation of bacterial superinfection, followed by formation of a biofilm that prevents eradication of infection. Bacterial degradation of biliary lipids then leads to precipitation of calcium bilirubinate and calcium fatty acid salts (144), which further obstructs the bile duct, causing biliary stasis and perpetuating infection. The presence of periampullary diverticula or biliary strictures is also a clinical risk factor, presumably because of bile stasis with bacterial overgrowth leading to CBD colonization (145).

In the Far East, brown pigment stones are common, often the sequelae of parasitic infection with *Clonorchis*. The major components of Eastern and Western brown pigment stones are derived from the products of bacterial degradation of biliary lipids: Calcium bilirubinate and calcium palmitate or stearate (134,146). Bilirubin is secreted as the highly water-soluble diglucuronide conjugate but can undergo degradation by bacterial β-glucuronidase to form UCB. Lacking the solubilizing hydrophilic glucuronides, UCB internally bonds to hydrogen and is extremely insoluble, precipitating as the calcium salt. The calcium bilirubinate in brown pigment stones is not polymerized as in black pigment stones. Bacterial degradation of biliary phosphatidylcholine by phospholipases produces lysolecithin and fatty acids. The latter, predominantly palmitic and stearic acid, precipitate as their calcium salts. Additionally, a protein matrix is composed of both mucin and bacterially derived glycoproteins. Scanning electron micrographs demonstrate bacteria in the core of brown pigment gallstones (147), and bacterial DNA is consistently present, attesting to the etiologic role of bacterial infection of bile.

Rarely, cholesterol stones can form as a primary event in the bile ducts (148). Abnormalities in cholesterol and bile duct metabolism may play a role in brown pigment stone formation (149). Patients with almost pure intrahepatic cholesterol stones have been reported to have impaired phosphatidylcholine secretion (143) and a mutation in MDR3, the phosphatidylcholine flippase (78).

Biliary Sludge

Biliary sludge, or microlithiasis, is thought to be a key intermediate step in both cholesterol and black pigment stone formation, although a minority of patients with sludge go on to develop stones (150). Sludge can be composed of either cholesterol crystals or bilirubin granules in a mucin matrix. The clinical outcome ranges from spontaneous dissolution and development of asymptomatic or

symptomatic stones to complications such as pancreatitis and biliary obstruction (151,152). Sludge formation has also been observed in patients receiving high doses of ceftriaxone, causing precipitation of the calcium salt (55), but resolution is rapid after discontinuation of the drug. In patients with cholesterol microlithiasis, cholesterol supersaturation was found to be due to phospholipid deficiency as opposed to an increased percentage of cholesterol in patients with gallstones (153), suggesting that at least in some cases microlithiasis may represent a disease process different than that typically leading to cholesterol cholelithiasis.

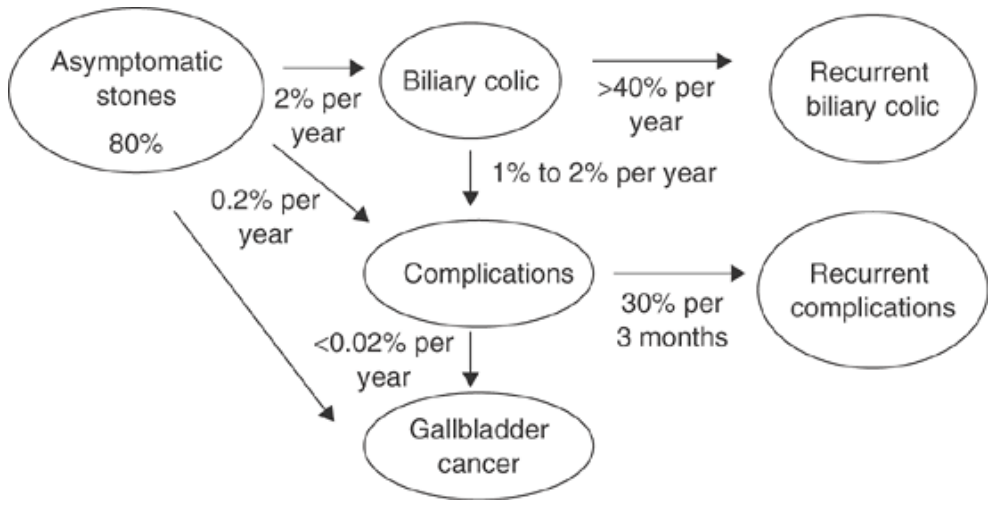
Clinical Spectrum of Gallstone Disease

Natural History of Gallstones

The clinical spectrum of gallstones may be divided into three stages: Asymptomatic, symptomatic, and complicated gallstone disease. Most gallstones are asymptomatic and remain so throughout the lifetime of the patient. Only between 15% and 20% of stones become symptomatic, between 1% and 3% per year (154,155) (Fig. 22.7). The most common initial symptom is biliary colic because gallstone disease rarely presents initially with complications. In some populations, such as the Pima Indians and South American natives, the rate of conversion to symptomatic disease appears to be higher (69). Once symptoms occur, the risk of recurrent symptoms is considerably higher: In one study, 70% of patients with symptoms in the previous year had recurrent symptoms within 2 years (156). In symptomatic patients, the risk of developing biliary complications is 1% to 2% per year, although symptomatic disease has a tendency to continue to remain so (154,155,156,157,158). Therefore, the natural history of asymptomatic stones is generally benign, whereas that of symptomatic stones follows a more aggressive course (156,159). These aspects are crucial in making clinical decisions about treatment of patients with gallstones (3,160).

The symptomatic stage of gallstone disease manifests itself primarily as attacks of biliary colic and episodes of pain, mainly in the epigastrium or right hypochondrium and often radiating to the back or the right shoulder or scapula (161,162,163). Biliary colic is believed to be caused by stone impaction in the cystic duct or gallbladder neck and produces a steady rather than a cramping pain, making the term biliary "colic" a misnomer. The pain correlates with gallbladder contractility (164). Lasting from 30 minutes to 6 hours, it arises and disappears slowly and may be associated

with nausea and vomiting. The pain typically occurs 15 minutes to 2 hours after eating but may be unrelated to food ingestion. Other features of dyspepsia, such as belching, bloating, bitter taste, or chronic pain should not be attributed to gallstones. Fatty food intolerance, commonly believed to be a characteristic of gallstones, is actually not more common in patients with gallstones than in others. The severity of biliary colic is sufficient to awaken the patient from sleep and often bring him or her to medical attention. Less commonly, this symptom complex may be associated with bile duct stones.



▪ **Figure 22.7** Natural history of gallstones. The 80% of stones that are asymptomatic tend to remain so. Most often their initial manifestation is biliary colic, rather than complications. However, once either biliary colic or complications occur, further symptoms are frequent. Gallbladder cancer is a rare complication.

The complicated stage of gallstone disease includes acute and chronic cholecystitis, acute pancreatitis, choledocholithiasis, ascending cholangitis, cholecystoenteric fistula, and gallbladder cancer. In acute cholecystitis, persistent cystic duct obstruction triggers an inflammatory process. The clinical picture resembles that of biliary colic, but with increased duration and severity, fever, leukocytosis, and often nausea and vomiting (165). On physical examination, right subcostal tenderness is characteristic, and pain and inspiratory arrest during palpation of the right subcostal area is relatively specific—Murphy's sign. Further complications include localized perforation, uncommonly, and free perforation and peritonitis, rarely.

Chronic cholecystitis is actually a pathologic condition and not clearly linked to a specific clinical entity. It is believed to be the result of recurrent episodes of biliary colic or acute cholecystitis, although a preceding history of attacks cannot always be obtained. The recurrent attacks or the presence of stones may contribute to local injury or ischemia and lead to a chronic inflammatory response with fibrosis, atrophy, and loss of gallbladder function.

Choledocholithiasis, or the presence of stones in the CBD, may be asymptomatic, but the likelihood of symptoms is held to be higher than that for gallbladder stones. In western countries these are typically secondary to stones in the gallbladder and occur in approximately 15% of patients with gallbladder stones (166). Their composition reflects that of the primary gallbladder stones, being composed mainly of cholesterol stones in the West (144). Primary stones in the biliary tree are more commonly seen in the Far East, particularly in the context of parasitic or bacterial infections in the bile ducts and are mainly of the mixed (brown stone) type (148). The natural history of choledocholithiasis is not well defined, but complications appear to be more frequent and severe than those with gallbladder stones (167). In contrast to gallbladder stones, the clinical presentation of choledocholithiasis is more likely to be extrahepatic biliary

obstruction or cholangitis. Elevation of alkaline phosphatase and γ-glutamyl transpeptidase levels is characteristic, as is mild jaundice with hyperbilirubinemia between 2 and 5 mg/dL but rarely more than 10 mg/dL (168,169). Although alkaline phosphatase and γ-glutamyl transpeptidase are the classic liver enzymes whose levels rise during cholestasis, in acute obstruction the transaminase levels may be significantly elevated (170). Choledocholithiasis may result in dilatation of the extrahepatic and intrahepatic bile ducts, although the lack of dilatation does not dissuade one from the diagnosis. Prolonged choledocholithiasis may lead to bile duct strictures and even secondary biliary cirrhosis (171).

Acute bacterial cholangitis is a medical emergency that is most often caused by a stone impacted in the distal common duct. The classic clinical presentation consists of biliary pain, jaundice, and fever (Charcot's triad). Chills and rigors are often seen, and, particularly in elderly patients, mental confusion, lethargy, and delirium may develop. Leukocytosis and jaundice with a bilirubin concentration of 2 mg/dL is seen in more than 80% of cases. The biliary tract becomes contaminated by bacteria, mainly *Escherichia coli*, *Klebsiella*, *Pseudomonas*, enterococci, or *Proteus*. Less commonly

anaerobes (e.g., *Bacteroides fragilis* and *Clostridium perfringens*) are involved. Bacterial translocation from the biliary tree to the bloodstream may lead to septicemia and septic shock. If untreated, bacterial cholangitis has a high mortality.

Biliary pancreatitis is believed to occur when a stone is impacted at the papilla of Vater, obstructing the outflow of the pancreatic duct (172). The obstruction may be transient, particularly during the spontaneous passage of a stone or of microcrystalline matter originating from the gallbladder. Often a direct association cannot be demonstrated, although systematic examination has demonstrated the presence of stones in the feces of up to 95% of patients after an episode of biliary pancreatitis (173). Microscopic examination of bile aspirated from the duodenum or CBD has been shown to reveal cholesterol monohydrate or calcium bilirubinate crystals in more than 60% of patients presumed to have idiopathic pancreatitis (152). The presence of sludge or small stones rather than larger stones may actually place patients at higher risk for pancreatitis (174). The clinical presentation and course of biliary pancreatitis is often indistinguishable from acute pancreatitis of any other cause. It is, however, crucial to identify those patients who have a stone impacted in the papilla because outcome in this condition is improved when they are treated by prompt endoscopic sphincterotomy and extraction of the stone (175,176).

Mirizzi's syndrome is a rare condition in which a stone becomes impacted in the cystic duct, causing external compression of the CBD with ensuing cholestasis (177). The clinical presentation may be indistinguishable from choledocholithiasis.

Gallbladder cancer is an uncommon complication of gallstone disease (178). In some populations, particularly in South Americans or Pima Indians, the frequency may be increased, but in most western countries, it is lower than the mortality during cholecystectomy and, therefore, does not constitute an indication for prophylactic surgical treatment of stones. Because the risk is highest for stones larger than 3 cm in diameter (179), cholecystectomy should be considered in such cases. Porcelain gallbladder is another rare condition defined by intramural calcification of the gallbladder wall. It is not necessarily associated with gallstones but has such a high predisposition for gallbladder cancer that it should

be considered an indication for prophylactic cholecystectomy.

Rarely, stones in the gallbladder may cause local injury and erode through the gallbladder wall into the intestines. The resulting cholecystenteric fistula most often communicates to the duodenum, although fistulization into the colon, stomach, and jejunum have been reported. If the stone is large (>25 mm in diameter), it may cause intestinal obstruction, in particular, at the level of the ileocecal valve. Gastric outlet obstruction due to a gallstone impacted in the distal stomach or duodenum is known as *Bouveret's syndrome* (180).

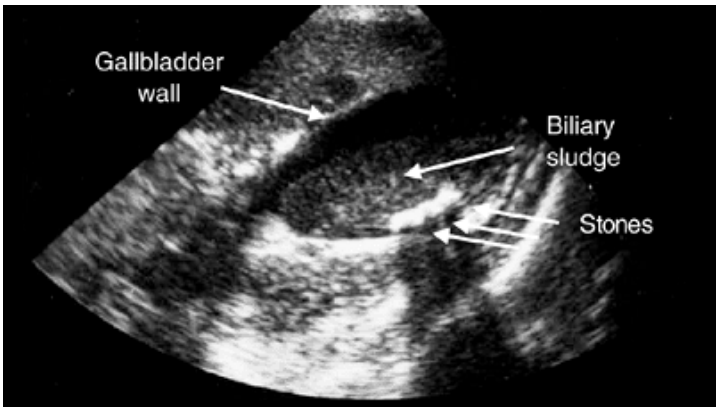
Diagnostic Modalities in Gallstone Disease

Imaging studies can reliably diagnose the presence and location of gallstones. Because gallstones are common in an older population, it is crucial for the clinician to decide whether abdominal symptoms are referable to the biliary tract before performing diagnostic studies. Otherwise, the incidental discovery of gallstones can lead to unnecessary intervention that is unlikely to relieve symptoms.

A plain abdominal radiograph rarely detects gallstones because most stones are radiolucent: Less than 25% contain enough calcium to be detected by radiographs. Only on rare occasions of air in the biliary tree (caused by surgery, endoscopic sphincterotomy, or spontaneous biliary enteric fistulation) can the plain abdominal radiograph be of assistance to the clinician.

Ultrasonography, on the other hand, has become the primary imaging modality in gallstone disease (Fig. 22.8). It is widely available, inexpensive, and completely noninvasive. Most gallstones these days are detected by ultrasonography, often as an incidental finding when evaluating the patient for an unrelated condition. Abdominal gas and obesity are limiting factors for the use of ultrasonography. Although operator dependent, the accuracy for detecting gallbladder stones, which appear as mobile echogenic foci casting an acoustic shadow, is in general more than 90%. The sensitivity of detecting stones larger than 2 mm in diameter exceeds 95%, with a specificity on the same order of magnitude (181). Ultrasonography can also detect sludge in the gallbladder. A sonographic Murphy's sign

(gallbladder tenderness under transducer pressure) is of value in diagnosing acute cholecystitis. Pericholecystic fluid is an additional quite specific indicator of this diagnosis. Despite its significant use in detecting gallbladder stones, ultrasonography even in the best hands has limited value in choledocholithiasis. Only approximately 25% to 40% of CBD stones are detected by transabdominal ultrasonography (182). This drawback is somewhat balanced by the ability of ultrasonography to recognize dilatation of the biliary tree beyond 7 mm, a value generally considered the upper limit of the normal choledochus.



▪ **Figure 22.8** Ultrasonographic demonstration of several gallbladder stones. A dependent layer of echogenic biliary sludge can be distinguished from the (*black*) echolucent bile above it. The stones cast a characteristic shadow, preventing further penetration of the ultrasound into the liver parenchyma.

Computed tomography (CT) scan has limited value in the diagnosis of gallstones for the same reasons as plain radiographs. However, CT scan improves the patient's evaluation by its capability of detecting or excluding complications, such as pancreatitis, pericholecystic fluid, perforation, or abscess formation. More recently, computerized analysis of CT scan data has been used to reconstruct bile duct images to provide a CT cholangiography (183).

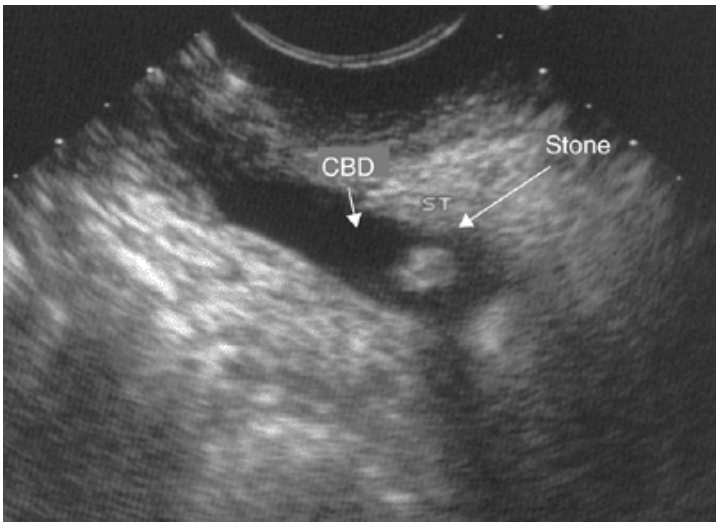
Oral cholecystography (OCG) was formerly used widely for diagnosing gallstones but has been largely superseded by ultrasonography. It is currently used for patients considered for oral dissolution therapy of gallstones because OCG can assess cystic duct patency and the cholesterol content of the stones. These two characteristics are prerequisites for this form of treatment.

Radionuclide scanning or cholescintigraphy after intravenous administration of a technetium-99m– labeled iminodiacetic acid derivative is valuable in assessing cystic duct obstruction in the diagnosis of acute cholecystitis and postoperative bile leaks (184). Nonvisualization of the gallbladder in the appropriate clinical setting is considered 95% sensitive and 80% to 90% specific for acute cholecystitis (181,185).

Magnetic resonance imaging (MRI) in its conventional form has little use in gallstone disease. However, magnetic resonance cholangiopancreatography (MRCP), a three-dimensional computer-generated reconstruction of the biliary system, is rapidly evolving as the most useful alternative for other methods of cholangiography (186). The method is noninvasive, does not require contrast agents, and can be done with an imaging time of only a few minutes (187). Moreover, the resolution is rapidly approaching a quality comparable to that of direct cholangiography (188). In the diagnosis of choledocholithiasis, MRCP has been reported to reach a sensitivity of around 95% (189,190). Hence, MRCP, where available, is turning into an increasingly important tool in the diagnosis of choledocholithiasis.

Endoscopic ultrasonography (EUS) is also becoming increasingly helpful in the

assessment of choledocholithiasis (Fig. 22.9), with a sensitivity and specificity of more than 95% (191,192). It is highly efficient in decreasing unnecessary endoscopic retrograde cholangiopancreatographys (ERCPs) (see subsequent text) (193). Moreover, it is superior to transabdominal ultrasonography in diagnosing small gallbladder stones, particularly in obese patients. It is also sensitive for detecting sludge and microcrystals in the gallbladder (194,195). EUS is, however, significantly operator dependent and unfortunately not routinely available in many centers.



▪ **Figure 22.9** Endoscopic ultrasonography of the common bile duct (CBD) demonstrating choledocholithiasis.

ERCP has been the gold standard for diagnosing choledocholithiasis for the last two decades (Fig. 22.10) (196). It is an invasive procedure associated with an inherent risk of pancreatitis. The current practice is shifting to MRCP and EUS as diagnostic tools, although ERCP remains the primary modality in managing choledocholithiasis, as discussed in subsequent text.

When gallstone disease is suspected but cannot be identified by other means, microscopic examination of duodenal contents aspirated after administration of CCK or bile obtained through an ERCP catheter may be employed to detect microcrystals. Cholesterol monohydrate crystals or calcium bilirubinate granules are readily recognized when the bile sediment is inspected under light microscopy, preferentially using polarized light (152,197). The presence of crystals is indicative of the presence of gallstone disease with sludge or small stones whose size may be below the limits of resolution of the imaging modalities (approximately 1 to 2 mm). The combination of EUS and bile analysis has been shown to be particularly sensitive (194).

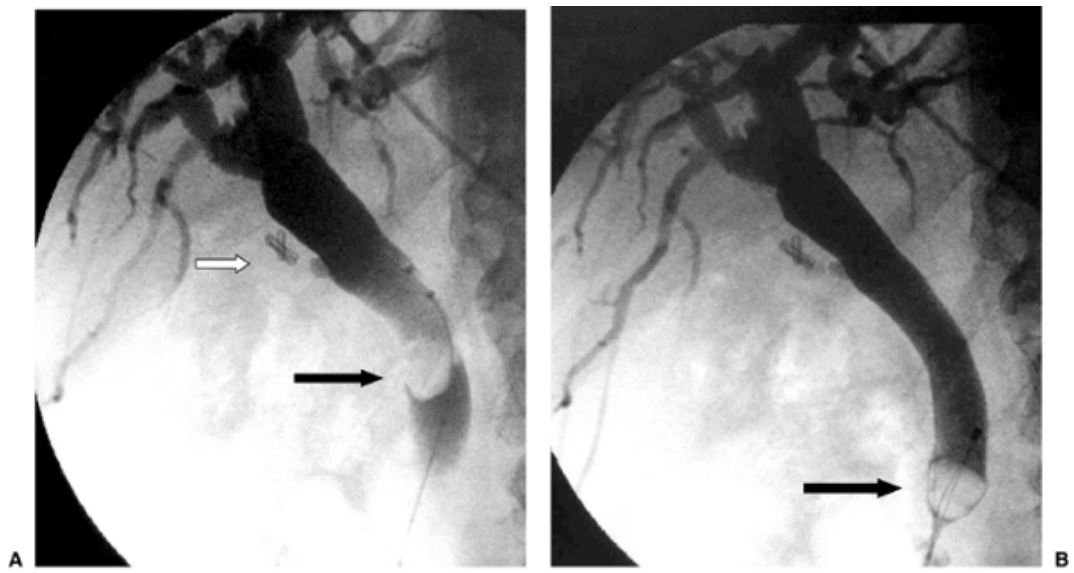
Management of Gallstones

Management of gallstones is guided by the natural history of the disease (Figs. 22.7 and 22.11) (2). Because

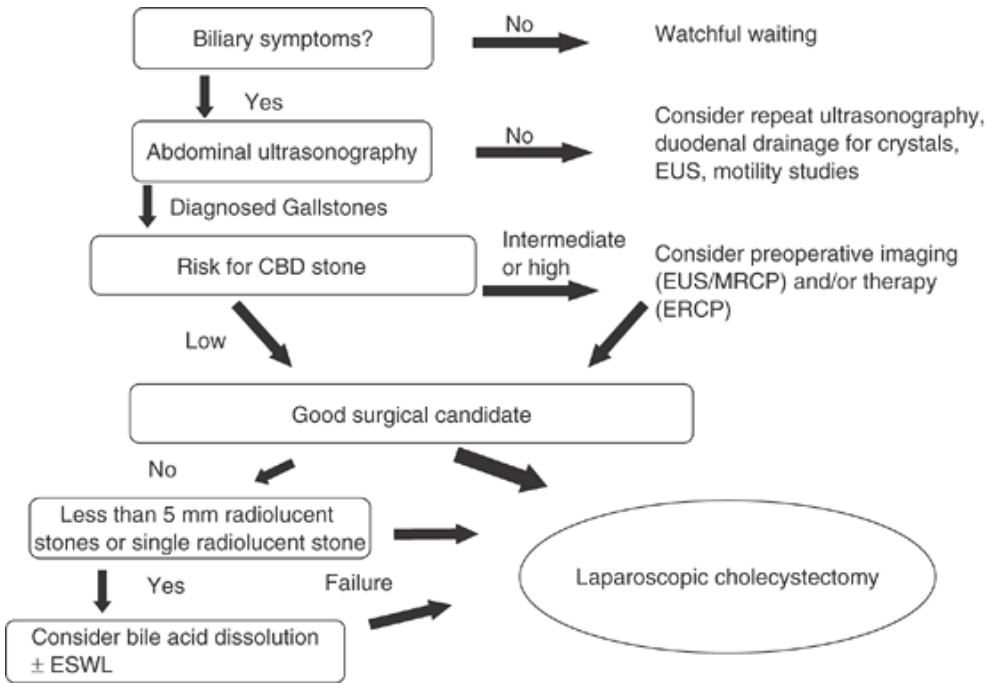
most stones are asymptomatic and will remain so indefinitely, the principal means of dealing with gallstones is expectant management, as supported by

decision analysis (198). However, the identification of patients with symptomatic stones rests on the clinical history rather than any diagnostic test. Patients with symptomatic stones, including biliary colic, should be offered therapy. Given the low likelihood of complications in patients with just pain, treatment is nonurgent (199). The mainstay of gallstone treatment is cholecystectomy. Removal of the gallbladder with the stones will cure the disease and prevent recurrence. In

circumstances in which surgery is too risky or rejected by the patient, alternative therapeutic options may be considered. Once a complication has occurred, the stones should be dealt with promptly.



▪ **Figure 22.10 A:** Endoscopic retrograde cholangiopancreatography of common bile duct stone (*black arrow*) identified after cholecystectomy (*white arrow* identifies surgical clips on the cystic duct). **B:** Endoscopic placement of a basket (*black arrow*) allows extraction of the stone.



▪ **Figure 22.11** Approach to diagnosis and treatment of gallstones. Details of management of complications of gallstones are described in the text. CBD, common bile duct; EUS, endoscopic ultrasonography; MRCP, magnetic resonance cholangiopancreatography; ERCP, endoscopic retrograde cholangiopancreatography; ESWL, extracorporeal shock wave lithotripsy.

Patients with classic symptoms of severe right–upper quadrant or epigastric pain are most likely to gain relief with cholecystectomy (161,162). The conditions of those with nonspecific symptoms such as dyspepsia, bloating, or flatulence are unlikely to be improved with cholecystectomy (200).

Surgical Treatment of Gallbladder Stones

The standard treatment for gallstones since 1892 has been cholecystectomy. Removal of the gallbladder under elective circumstances is associated with very low morbidity and mortality (201,202). In the early 1990s, the field underwent a revolution, when the conventional procedure was replaced by laparoscopic cholecystectomy. First introduced in 1987, the laparoscopic approach reduced postoperative pain, allowing patients to be discharged within 1 to 2 days of surgery and rapidly return to their normal activities (203). Less than 5% of laparoscopic cholecystectomies are converted to open procedures in the operating room because of excessive inflammation, adhesions, unexpected findings, or anatomic variations (204,205). Laparoscopic cholecystectomy is associated with a decreased risk of mortality, cardiopulmonary complications, and wound infections, but an increased risk of bile duct injuries (204,206). Initially believed to be part of the learning curve, it has become evident that the rate of bile duct injury remains between 0.2% and 0.5%, even in the most experienced hands (207). Because the advent of laparoscopy has also increased the rate of cholecystectomies performed by 20% to 30% (208), bile duct injuries are not an uncommon complication seen by gastroenterologists or hepatologists.

The most common immediate problem is bile leak, often manifested by postoperative pain and fever, and is treated easily by prompt endoscopy (209). At ERCP a stent is placed with or without sphincterotomy to decrease outflow resistance and enable spontaneous closure of the leak, usually within a few days. Late complications may be more severe and usually result from bile duct stricturing. Patients may develop asymptomatic cholestasis or jaundice and cholangitis with a risk of secondary biliary cirrhosis if untreated. Endoscopic treatment with balloon dilatation and stent placement is usually effective (210), but specialized surgical repair may be required, particularly in proximal injuries. Although most bile duct injuries are treated efficiently by endoscopy or surgery, they were shown to have a negative effect on overall patient survival (211). Intraoperative cholangiography has been suggested to reduce the risk of bile duct injury, with variable results (211,212). Minilaparotomy has been advocated by some to reduce the risks while retaining the benefits of laparoscopic surgery (213).

Timing of surgery in the context of acute cholecystitis has been debated. It was thought that surgery would increase surgical mortality during the acute episode and should, therefore, be deferred to 6 to 8 weeks after recovery from the event. However, when studied prospectively, it appeared that early cholecystectomy within days of presentation is actually associated with less morbidity and mortality and can also be performed safely through laparoscopy (214). Prompt cholecystectomy is particularly indicated in patients with diabetes and elderly patients, who are more likely to develop serious complications from the acute episode. Patients with acute cholecystitis who are too unstable to tolerate general anesthesia and full cholecystectomy will improve if the gallbladder is decompressed by way of a percutaneous or surgical cholecystostomy (215).

Removal of the gallbladder typically does not alter digestive function measurably. Approximately 5% of patients will experience increased stool frequency or diarrhea postcholecystectomy, possibly related to increased enterohepatic cycling of the bile salt pool (216). Administration of bile acid binding resins such as cholestyramine is often sufficient to control diarrhea, which usually improves over time (217). Recently, some concern has been raised about an increased risk of intestinal cancer after cholecystectomy (218,219). At most, the risk is only modestly elevated, predominantly in women (220).

Nonsurgical Treatment of Gallbladder Stones

Despite the efficient removal of gallbladder stones by surgery, approximately 20% of patients continue to suffer from pain after cholecystectomy (221). Moreover, because nonsurgical expectant management carries a low risk of complications, it can be a reasonable option for mildly symptomatic patients (222).

Oral bile acids can dissolve cholesterol gallstones (223,224). Initial efforts employed chenodeoxycholic acid (chenodiol), a primary bile acid of humans. Chenodiol is a strong detergent whose primary mechanism of action is enhancing the micellar lipid solubilizing capacity of bile. Side effects related to its detergency included diarrhea and hepatocellular injury. Subsequently ursodeoxycholic acid (UDCA), a primary bile acid of bears and normally present only in minute amounts in human bile, was found to have equal efficacy for gallstone dissolution, with virtually no toxicity. UDCA also reduces biliary cholesterol secretion, mainly through its inhibitory effect on intestinal cholesterol absorption (225). Although UDCA is not

a good detergent in allowing true cholesterol solubilization, cholesterol is effectively dispersed in bile as vesicles, thereby preventing and reversing cholesterol crystallization (226).

The main limitation of oral dissolution therapy is that its efficacy is limited to a fraction of patients with cholesterol stones (15% or less) (227). Only small cholesterol stones (preferably <5 mm in diameter) in a functioning gallbladder are amenable to bile acid dissolution. The OCG is the best tool for diagnostic evaluation and patient selection. Small stones that form a floating layer in the gallbladder are composed of almost pure cholesterol and have approximately a 75% likelihood of dissolution (228). If OCG is unavailable, CT scan can be used to select stones that have high cholesterol content (isodense with bile or less than 100 Hounsfield units), whereas gallbladder function may be verified by ultrasonography (before and after gallbladder stimulation) or radionuclide scanning.

Desaturation of bile is obtained with UDCA at a dose of 8 to 12 mg/kg per day. The entire dose may be given at bedtime to optimize desaturation of bile during sleep. Treatment typically requires between 12 and 24 months and should be continued 6 months beyond successful dissolution. Response should be assessed after 6 months, and treatment of nonresponders, that is, those with no decrease in gallstone size, should be discontinued because of the high likelihood of further failure. After successful dissolution, gallstones recur in approximately 50% of individuals over a period of 5 to 10 years (229).

Oral gallstone dissolution therapy is a reasonable option only for symptomatic patients with gallbladder sludge or small cholesterol stones that have not caused complications. UDCA therapy has been associated with a reduction in the occurrence of biliary colic in asymptomatic and symptomatic patients (230). Dissolution of cholesterol crystals within biliary sludge has been suggested as a possible mechanism for this effect. Other oral agents, such as monoterpenes, or HMG-CoA reductase inhibitors, have limited efficacy, mainly in combination with UDCA (231). Recently, fatty acid bile acid conjugates have been shown to dissolve cholesterol gallstones in an animal model (232). These and other comparable agents may prove useful in humans.

A complementary nonsurgical option is extracorporeal shock wave lithotripsy (ESWL) (233), which is actually an extension of oral gallstone dissolution therapy. By fragmenting stones with external shock waves by the same principles as urinary lithotripsy, larger cholesterol stones can be brought into the size range (<5 mm in diameter) ideal for bile acid dissolution. Some fragments are expelled from the gallbladder even without dissolution. ESWL works best with solitary cholesterol gallstones smaller than 2 cm in diameter. The major complications of ESWL are biliary colic and acute pancreatitis resulting from expulsion of gallstone fragments. Because of the inclusion limitations (larger single cholesterol stones in a functioning gallbladder), only 16% of symptomatic patients with gallstones are candidates for this form of therapy (227). The recurrence rate (around 30% within 5 years) seems to be somewhat lower than that with dissolution therapy alone (234). ESWL has been approved for general use in the United States but has been used more frequently in Europe.

A third nonsurgical option employs detergents or organic solvents instilled directly into the gallbladder. Methyl-*t*-butyl ether (MTBE) has been instilled through a percutaneously placed transhepatic catheter curled in the fundus of the

gallbladder to achieve dissolution of cholesterol gallstones within hours in more than 90% of cases (235). The major risks of the procedure are hemorrhage after liver puncture, persistent bile fistula, or inadvertent spilling of solvent into the duodenum (leading to nausea and vomiting) or blood (leading to renal failure or death) (235). Because of the relative invasiveness and high recurrence rate, contact solvent dissolution is performed in only a handful of centers worldwide.

Treatment of Bile Duct Stones

Gallstones in the CBD may be identified preoperatively or postoperatively when imaging is indicated by symptomatology or at the time of surgery through intraoperative cholangiography or cholangioscopy. They should be treated for the reasons mentioned earlier. CBD stones were traditionally removed by opening and exploring the CBD at the time of cholecystectomy, after which a T tube is left in place for 3 to 7 days to facilitate healing. Open common duct exploration prolongs surgery and convalescence and increases surgical morbidity and mortality substantially, whereas laparoscopic bile duct exploration although feasible is technically demanding and performed only in a minority of centers (236,237). Hence, endoscopic management is preferred in most clinical situations.

Endoscopic treatment is usually done under conscious sedation as an outpatient procedure (238). During ERCP the papilla is cannulated and stones are extracted with a balloon or a Dormia basket (Fig. 22.9). A sphincterotomy is usually performed to facilitate stone removal, although for small stones this may be unnecessary or substituted by sphincteroplasty (transient balloon dilatation) to conserve sphincter function (239). Endoscopic sphincterotomy is associated with a 5% to 10% risk of complications such as pancreatitis, hemorrhage, or perforation and occasional late stenosis (240). Conventional ERCP is successful in removing most (>85%) CBD stones. Large (>18 mm in diameter) stones may require fragmentation before removal. This is routinely done by a mechanical lithotriptor

through the endoscope. More sophisticated tools, such as contact lithotripsy by pulse laser or shock waves or ESWL, increase the success rate to approximately 90% (241). Temporary or long-term endoscopic stenting may be offered for difficult cases or in high-risk patients. Urgent endoscopic drainage is the treatment of choice and is often lifesaving for patients with ascending bacterial cholangitis (242).

Since the advent of laparoscopic cholecystectomy, the timing and means of treating concomitant gallbladder and bile duct stones have been debated extensively (243). Theoretically, there are several possible advantages of the laparoscopic one-stage procedure, but this is often unavailable and there are no concrete data to support the advantages (244). Therefore, choledocholithiasis is usually treated endoscopically to avoid open surgery and the associated morbidity (245). However, extensive use of preoperative ERCP may lead to unnecessary endoscopies and cases of pancreatitis, whereas postoperative ERCP may fail and require reoperation. Intraoperative ERCP is possible, but usually impractical. A reasonable strategy is for patients with a high risk of choledocholithiasis to undergo preoperative ERCP while those with moderate risk can have intraoperative cholangiography and laparoscopic CBD exploration (246). Although the approach should be based on local expertise and preferences, most centers perform a preoperative ERCP when the likelihood of choledocholithiasis is high. A

dilated CBD with a stone seen on ultrasonography, persistent jaundice, and elevated alkaline phosphatase levels are good predictors of choledocholithiasis (247,248). In less clear cases, MRCP and EUS can be used to guide therapeutic decisions. A recent decision analysis in patients with acute biliary pancreatitis found MRCP and EUS to be most useful for patients with an intermediate probability of choledocholithiasis (249). When the likelihood of choledocholithiasis is low, preoperative ERCP is best avoided (250). When unexpected choledocholithiasis is revealed intraoperatively, a transpapillary guidewire, catheter, or endoprosthesis may be left in place by the surgeon to increase the postoperative success rate of ERCP (251). Occasionally, small stones will pass spontaneously and uneventfully, rendering ERCP unnecessary (252).

Percutaneous transhepatic cholangiography (PTC) has limited use in the diagnosis or management of gallstones, except as rescue therapy for patients with choledocholithiasis in whom ERCP has failed. PTC provides effective biliary drainage in the setting of acute cholangitis and enables subsequent successful ERCP by the combined percutaneous and endoscopic approaches (253). A guidewire is introduced percutaneously through the bile ducts into the duodenum and is used to cannulate the bile duct to facilitate sphincterotomy and stone extraction by standard means. If ERCP is unavailable, the more invasive transhepatic route may be used for stone extraction.

If patients are at high surgical risks and stones in the common duct can be cleared by endoscopy, it may be reasonable to defer cholecystectomy. Several retrospective and prospective nonrandomized studies have shown that less than 15% of patients develop biliary complications during follow-up (254). Patients with a patent cystic duct at surgery seem to fare particularly well (255). However, a more recent prospective randomized trial has shown that up to 47% of patients may develop biliary symptoms (256). Therefore, whenever possible, surgery should be considered the definitive therapy to minimize recurrent biliary symptoms (257).

Treatment of Microlithiasis

Patients with microlithiasis or biliary sludge comprise a special subgroup of patients who may also develop attacks of biliary pain, acute pancreatitis (152), or cholecystitis (55). Microlithiasis is believed to represent an early stage of gallstone disease because, when left untreated, approximately 8% will develop overt gallstones (150). Most often, the sludge will disappear spontaneously, particularly in circumstances of transient lithogenicity such as pregnancy, parenteral nutrition, or rapid weight loss (101,258). Persistent microlithiasis can be treated by oral bile acid therapy, although significant symptoms and complications should be treated by cholecystectomy or endoscopic sphincterotomy, similar to the approach for macroscopic stones (55).

Prevention of Gallstones

Because of the high prevalence of gallstones and the high costs involved in their management, the ultimate approach should be prevention. Unfortunately, most attempts to effectively prevent gallstone formation in the general population have failed. The increased recognition of risk factors for gallstone formation has unfortunately not resulted in better means of prevention. Despite recommendations for low-fat, low-calorie diets and moderate alcohol intake with regular physical activity, the prevalence of gallstones has not declined.

There are, however, some high-risk situations in which prevention is possible. During rapid weight loss, in particular, after bariatric surgery, prophylactic administration of UDCA will reduce the likelihood of gallstone formation from approximately 30% to almost nil (108). In patients receiving TPN, the administration of CCK (259) or early limited enteral feeding to induce gallbladder contraction may prevent the formation of biliary sludge and stones. More widespread preventive measures will hopefully be available as our understanding of the pathophysiology of gallstones progresses and more effective therapeutic agents are discovered.

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Chapter 23
Primary Sclerosing Cholangitis

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Key Concepts

- Primary sclerosing cholangitis (PSC) is a progressive, cholestatic liver disease that is characterized by diffuse chronic inflammation and fibrosis of the biliary tree.
- PSC affects primarily young to middle-aged men and frequently is associated with inflammatory bowel disease (IBD), most often chronic ulcerative colitis (CUC).
- The cause of PSC remains unknown, although the interaction of exogenous factors with the genetically predisposed individual is likely critical in disease pathogenesis.
- Cholangiocarcinoma is the most feared complication of PSC; however, colon cancer complicating CUC in a patient with PSC is important as well.
- The prognosis of PSC is variable, but in general the disease process is progressive.
- To date, no medical therapy is available benefit although a variety of pharmacologic agents have been tested; nevertheless, high-dose ursodeoxycholic acid deserves further study in the context of randomized controlled trials.
- Liver transplantation is the best available treatment option for patients with advanced PSC; however, the disease can recur in 20% of recipients after successful transplantation.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown causation that is frequently associated with inflammatory bowel disease (IBD). The disorder is characterized by diffuse inflammation and fibrosis of the biliary tree and usually leads to biliary cirrhosis, which can be complicated by portal hypertension and hepatic failure.

PSC was first described by Delbet in 1924 (1). Before the widespread availability of endoscopic retrograde cholangiopancreatography (ERCP) in mid-1970s, PSC was considered a rare disease (2). At present, PSC is seen as an important cause of chronic cholestasis in adults. However, it is still unclear whether the prevalence of the disease has increased in recent decades. The greater availability of ERCP and magnetic resonance cholangiopancreatography (MRCP) as well as the recognized association of PSC with IBD along with screening of these

patients with liver tests have probably enhanced the frequency of PSC diagnosis. To date, we have better understanding of the natural course of PSC, although the cause and identification of specific beneficial therapies have eluded investigators so far.

Epidemiology

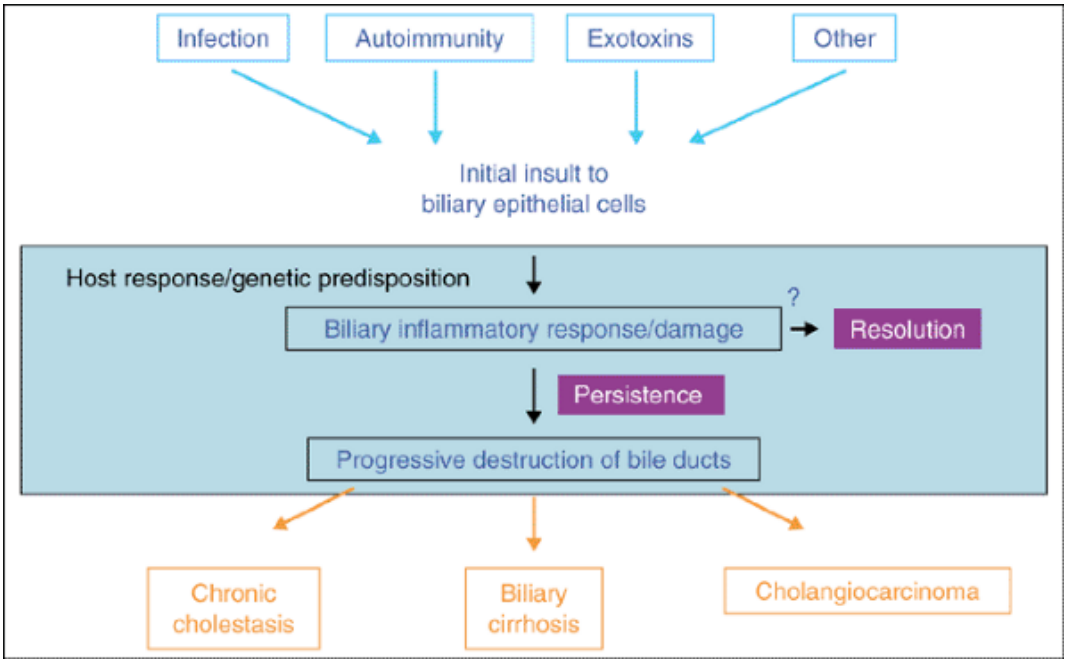
Two recent epidemiologic studies from the United States and United Kingdom described the incidence and prevalence of PSC in the community setting. The first study from Olmsted county, Minnesota, estimated the age-adjusted incidence of PSC to be 0.9 per 100,000 individuals with point prevalence of 13.6 per 100,000 persons in 2000 (3). Specifically, the age-adjusted incidence was 1.25 and 0.54 per 100,000 men and women, respectively (3). The estimated prevalence was 20.9 per 100,000 for men and 6.3 per 100,000 women (3). On the basis of this study, it was projected that

approximately 29,000 cases of PSC exist in the white USA population. Moreover, the median age at PSC diagnosis was 40 years, 68% (15 of 22) of the patients were men and 73% (16 of 22) of the patients had concurrent IBD (3).

The second study from the city and county of Swansea, Wales, UK reported an annual incidence of 0.91 per 100,000 and a point prevalence of 12.7 per 100,000 individuals (July 1, 2003) (4). The median age at PSC onset was 52 years, 62% (33 of 53) of the patients were men, and 62% (33 of 53) of the patients had coexisting IBD (4). Although these two studies have shed light on the epidemiology of the disease, additional population-based studies are required to better define the prevalence and natural history of PSC, a chronic cholestatic liver disease that leads not only to hepatic failure but also predisposes to malignancies of the colon and liver.

Pathogenesis

To date, the exact pathogenesis of PSC remains unknown, although a number of avenues have been explored over the past three decades. A consensus pathogenesis postulates that PSC develops as the result of acquired exotoxins, infectious agents or autoimmunity interacting with predisposing host factors (Fig. 23.1). It is proposed that this interplay leads to an initial damage of cholangiocyte, the target epithelial cell that lines the bile ducts. Subsequently, a biliary inflammatory response takes place but most individuals likely recover (i.e., resolution) without consequences. However, it is the genetic predisposition of the host and probably other unknown mechanisms that contribute to persistance of inflammation of the bile ducts resulting in progressive biliary destruction and complications of PSC such as chronic cholestasis, biliary cirrhosis, and cholangiocarcinoma (Fig. 23.1).



▪ **Figure 23.1** Proposed pathogenesis of primary sclerosing cholangitis.

Genetic Factors

Several observations are consistent with an important role for genetic factors. First, familial PSC cases have been reported (5). Second, a recent report indicated the increased prevalence of PSC among first-degree relatives of patients who are affected (6). These authors calculated an almost 100-fold relative risk of developing PSC in families with an affected member, thereby supporting the presence of inherited elements in disease pathogenesis. In addition, several case control studies have demonstrated the genetic predisposition to PSC development by identifying genetic variants associated with patients compared to controls. To this extent, Chapman first reported the HLA B8 frequency to be significantly higher in patients with PSC (60%) compared with controls (25%) ($P < 0.001$) (7). Subsequently, Donaldson demonstrated an association of PSC with the HLA-A1, B8, DR3 haplotype (40% in cases vs. 12% in controls, $P < 0.0005$) (8).

Moreover, in the past 5 years, genetic polymorphisms (i.e., DNA variants) associated with susceptibility to PSC have been reported including (i) the promoter region of the tumor necrosis factor-α (TNF-α) receptor (9); (ii) stromelysin (i.e., matrix metalloproteinase 3) (10); (iii) major histocompatibility complex class I related-MIC gene family (MICA) gene (i.e.,

MICA) (11); (iv) CCR5-Delta 32 mutation (12); and (v) intracellular adhesion molecule-1 ICAM-1 (i.e., intracellular adhesion molecule-1) (13) (Table 23.1). From a theoretical standpoint, the variety of susceptible genes interacting with environmental factors likely explain the heterogeneity of PSC phenotype as this relates to disease development, progression and complications. It is expected that many genetic variants (i.e., susceptible alleles) predispose to disease, each contributing a small effect on the PSC phenotype. To this end, large association

(i.e., case-control) and familial studies are needed to better dissect the genetic susceptibility of PSC before we develop novel diagnostic tools and intervening therapies.

Table 23.1. Susceptibility Genes in Primary Sclerosing Cholangitis

Gene	Type	Variation	Reference
TNF-α	Cytokine	G/A, substitution	(9)
Stromelysin	Matrix metalloproteinase	5A/6A alleles	(10)
MICA	MHC molecule	*002/*008 alleles	(11)
CCR5-Delta 32	Chemokine	32 base pair deletion	(12)
ICAM-1	Adhesion molecule	K469E	13

TNF-α, tumor necrosis factor α; MICA, major histocompatibility complex class I related-MIC gene family; MHC, major histocompatibility complex; CCR5-Delta 32, chemokine, CC motif receptor 5; ICAM-1, intracellular adhesion molecule-1.

Other Proposed Hypotheses of Primary Sclerosing Cholangitis Pathogenesis

Because of the finding that IBD coexists in approximately 75% of patients with PSC, much interest has been paid to the potential role of an inflamed colon in causing the liver disease (14). For long it was believed that inflammation of the colon may increase the colonic permeability to various intraluminal products leading to liver injury. To this extent, bacteria or bacterial toxins have been considered but have not been convincingly demonstrated to play a pathogenetic role in PSC (15,16). In addition, abnormal bile acids generated by bacterial action in the diseased colon and directly absorbed through the colonic mucosa into the portal system have been suggested as a possible etiology of PSC. Nevertheless, no direct evidence in support of this theory has been forthcoming (17).

In an animal model, inflammatory bacterial peptides led to portal inflammation and histologic changes suggestive of PSC (18,19). In this model, a variety of agents were useful in blocking this response, including an inhibitor of tumor necrosis factor (TNF). However, when pentoxifylline, a TNF inhibitor, was used in patients with PSC, no beneficial effect was found, and doubt was cast on the value of this model in understanding the disease in humans (20). Furthermore,

the finding that PSC can develop in approximately 25% of patients without concurrent IBD, the lack of association between the severity of the colonic inflammation and the likelihood of PSC development and the fact that proctocolectomy for chronic ulcerative colitis (CUC) does not affect the natural history of PSC (21) speak against an essential role of the inflamed colon in development of the cholestatic liver disease.

At present, there is no convincing evidence of a virus or other microorganism causing PSC. The usual hepatotrophic viruses (i.e., hepatitis A virus [HAV], hepatitis B virus [HBV], and hepatitis C virus [HCV]) have been excluded. Cytomegalovirus can produce changes suggestive of PSC in patients with acquired immunodeficiency states, but in patients with competent immune function, no evidence of cytomegalovirus infection has been found (22). Reovirus type 3 was considered a possible causative agent but further work excluded it as an etiology for PSC (23).

Table 23.2. Diseases Associated with Primary Sclerosing Cholangitis

Inflammatory bowel disease
Autoimmune hepatitis
Chronic pancreatitis
Celiac disease
Rheumatoid arthritis
Retroperitoneal fibrosis
Peyronie's disease
Riedel's thyroiditis
Bronchiectasis
Sjögren's disease
Glomerulonephritis
Systemic lupus erythematosus
Pseudotumor of the orbit
Vasculitis
Autoimmune hemolytic anemia
Immune thrombocytopenic purpura
Angioimmunoblastic lymphadenopathy
Histiocytosis X
Cystic fibrosis
Sarcoidosis
Systemic mastocytosis
Polymyositis
Alopecia universalis
Thymoma
Ankylosing spondylitis

Immune-mediated damage of bile ducts seems a plausible mechanism leading to PSC. Abnormalities of the humoral immune system in PSC include the presence of (i) hypergammaglobulinemia, particularly elevated immunoglobulin M levels, (ii) circulating immune complexes; and (iii) activated complement (24). Moreover, patients with PSC have serum positivity for several auto-antibodies including antineutrophil cytoplasmic antibodies (ANCA), anticardiolipin antibodies and

antinuclear antibodies (53%) (25). In addition, the cellular immune system in PSC can be abnormal as indicated by the decrease in the total number of circulating T cells caused due to a decline in CD8 (suppressor/cytotoxic cells) and an increase in circulating B-cells. On the other hand, the documented aberrant expression of HLA class II antigens on the biliary epithelial cells may serve to target an immune response against the biliary cells; (26,27) Although the presence of ICAM-1, which serves as a ligand for the leukocyte function-associated antigen (LFA-1), may help form connections between T-lymphocytes and antigen-presenting cells. Indeed, increased levels of ICAM-1 have been found in both the bile duct epithelial cells and serum (27,28,29). LFA-1 also appears to be overexpressed by intrahepatic lymphocytes, but this expression may simply be induced by proinflammatory cytokines (30). Despite all these observations, the documented altered immune status may simply be an epiphenomenon and yet not linked directly to PSC pathogenesis.

Clinical Features

PSC affects men almost twice as commonly as it does women and the average age at diagnosis is the early 40s (3). In the past two decades, a frequent clinical scenario of diagnosis includes patients who are asymptomatic and come to medical attention solely because of abnormal liver tests. Contrary symptoms of advanced stages of PSC such as jaundice, pruritus, fever, or manifestations of portal hypertension are less common as initial presentation. Physical examination may be unrevealing. Hepatomegaly, splenomegaly, hyperpigmentation, and skin excoriation can be found. Health-related quality of life is significantly impaired among patients with PSC similar to that found in other chronic liver diseases, such as primary biliary cirrhosis (PBC) and chronic viral hepatitis. (31,32).

PSC usually affects the entire biliary tree. Approximately 20% of patients have involvement of the intrahepatic bile ducts alone and approximately 5% of patients have disease involving the interlobular and septal bile ducts (i.e., *small-duct PSC*) seen only in liver biopsy specimens while ERCP is normal (33,34,35,36). Another clinical entity is the *overlap syndrome* in which PSC and autoimmune hepatitis coexist and occurs in approximately 5% of patients (33,34,35).

PSC afflicts children rarely (37). In this population, the disease seems to have more frequent features of autoimmune hepatitis (38,39).

Laboratory Findings

Biochemical Testing

The biochemical findings of patients with PSC are nonspecific. Neverthelesss, chronic biochemical cholestasis is apparent. Alkaline phosphatase is the most commonly elevated liver enzyme; however, the occasional patient with well-documented PSC but normal alkaline phosphatase levels has been described (40). Aminotransferase levels frequently are elevated and the degree of elevation is usually only modest except in patients with *overlap syndrome* in whom these levels could be markedly increased. Serum bilirubin is typically normal but in patients with advanced disease can reach very high levels.

Immunologic Testing

Overall, there are no immunologic or autoantibody profiles specific for PSC.

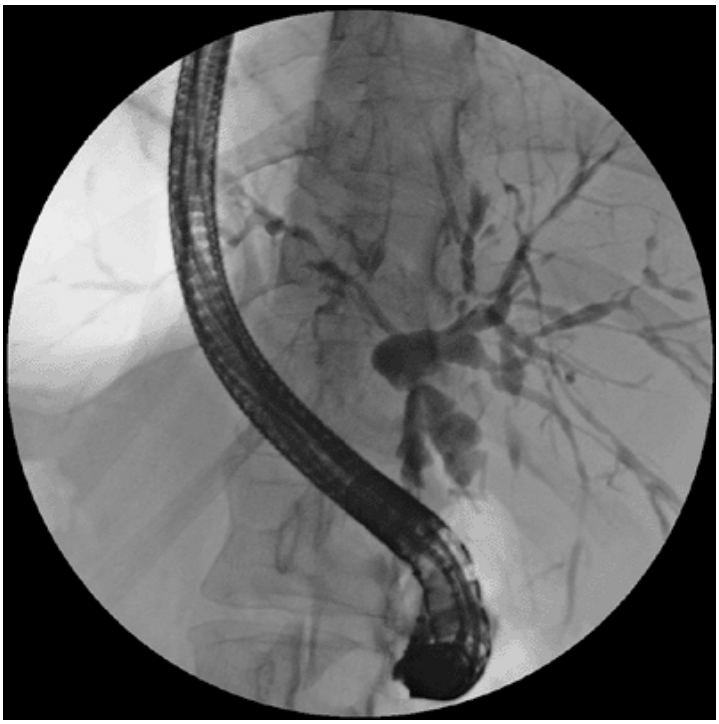
Hypergammaglobulinemia occurs in approximately 25% of patients with PSC and immunoglobulin M is the most commonly elevated component. PSC patients have serum positivity for several auto-antibodies including ANCA (84%), anticardialipin antibodies (66%) and antinuclear antibodies (53%) (25). Antimitochondrial antibodies are rare in patients with PSC.

Imaging Studies

Visualization of the biliary tree is essential for establishing the diagnosis of PSC. ERCP has been the diagnostic procedure of choice, although MRCP is almost equally sensitive and specific for detection of the disease. Additionally, MRCP is more cost-effective for establishing the diagnosis in patients with suspected PSC (41,42,43). Percutaneous transhepatic cholangiogram (PTC) can be used to evaluate the bile ducts for PSC but because of the frequently sclerotic intrahepatic biliary system gaining access through the percutaneous route could be challenging.

Typical cholangiographic findings of PSC include multifocal stricturing and beading, usually involving both the intrahepatic and extrahepatic biliary system (Fig. 23.2). Involvement of the intrahepatic tree alone can be found in as many as 20% of patients (44,45). Often the strictures are diffusely distributed, short

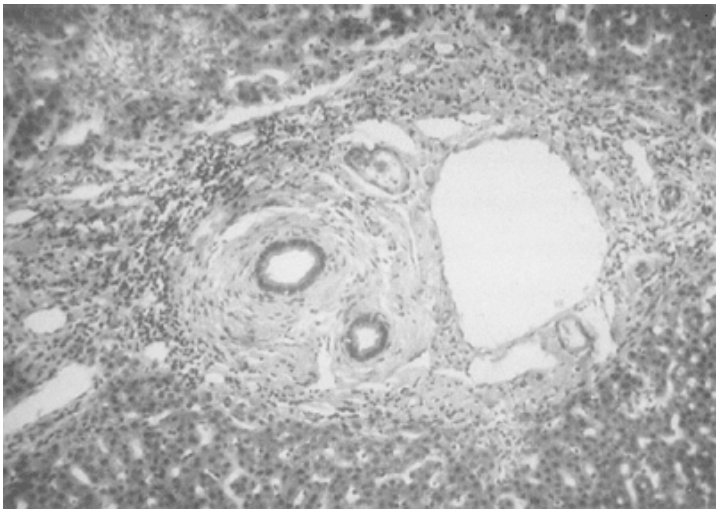
in length and annular. Cystic duct and gallbladder involvement are present in as many as 15% of patients (46). During cholangiography, the presence of polypoid masses should raise the suspicion of cholangiocarcinoma, although the diagnosis of the latter can be very difficult to establish.



▪ **Figure 23.2** ERCP of a patient with primary sclerosing cholangitis. Extensive extrahepatic and intrahepatic biliary disease is evident.

Histology

Liver biopsy findings are usually not enough to establish the diagnosis of PSC. The classic *onion-skin* fibrosis is present in fewer than 10% of biopsy specimens obtained from patients with PSC, but when seen is almost pathognomonic (Fig. 23.3) (47). The histologic grading system, proposed by Ludwig et al. (47), has four stages: Stage 1, portal; stage 2, periportal; stage 3, septal; and stage 4, cirrhosis (Table 23.3). Of interest, in patients with PSC, the histologic changes seem to be quite varied in different segments of the same liver at any given point in time. To this end, histologic staging has been avoided as a component of the most recent survival model of PSC (48).



▪ **Figure 23.3** The "onion-skin fibrosis" lesion is characteristic of primary sclerosing cholangitis but is not typically found in liver biopsy specimens from patients.

Table 23.3. Histologic Staging of Primary Sclerosing Cholangitis

Portal stage (stage 1)	Portal edema, inflammation, ductal proliferation; abnormalities do not extend beyond the limiting plate.
Periportal stage (stage 2)	Periportal fibrosis, inflammation with or without ductular proliferation; piecemeal necrosis may be present.
Septal stage (stage 3)	Septal fibrosis or bridging necrosis can be identified.
Cirrhotic stage	Biliary cirrhosis.

(stage 4)	

Diagnostic Criteria and Differential Diagnosis

The diagnostic criteria for PSC were first formulated nearly 40 years ago (49). These criteria included (i) absence of previous surgical trauma to the biliary tree; (ii) lack of stones in the gallbladder and common bile duct; (iii) stenosis/stricturing involving most of the biliary system; and (iv) exclusion of biliary malignant disease. Because of the advent of ERCP in the mid-1970s, the diagnosis can be made without surgery. More recently, the availability of MRCP as a screening test for patients with suspected PSC made noninvasive diagnosis possible (41,42,43).

At present, the diagnostic criteria for PSC include (i) typical cholangiographic abnormalities involving any part of the biliary tree; (ii) compatible clinical and biochemical findings (i.e., cholestasis for more than 6 months); and (iii) exclusion of other causes of secondary sclerosing cholangitis (Table 23.4). Liver biopsy has been used in the past to help confirm the diagnosis, although the specificity and sensitivity of the biopsy have come under question. Histologic findings are not always found of value in the most recently developed prognostic scoring systems for patients with PSC (48,50,51,52). However, liver biopsy is useful in the care of a patient with suspected PSC but normal cholangiographic findings (i.e., *small-duct PSC*) and in the setting of a patient with *overlap syndrome*. The differential diagnosis of PSC is outlined in Table 23.4.

Table 23.4. Diagnostic Criteria for Primary Sclerosing Cholangitis

Typical cholangiographic abnormalities involving any part of the biliary tree
Compatible clinical (cholestatic symptoms, history of inflammatory bowel disease) and biochemical findings (twofold to threefold increase in serum alkaline phosphatase level for longer than 6 m)
Exclusion of identifiable causes of secondary sclerosing cholangitis:
AIDS cholangiopathy
Bile duct neoplasm (unless diagnosis of PSC previously established)
Biliary tract surgery, trauma

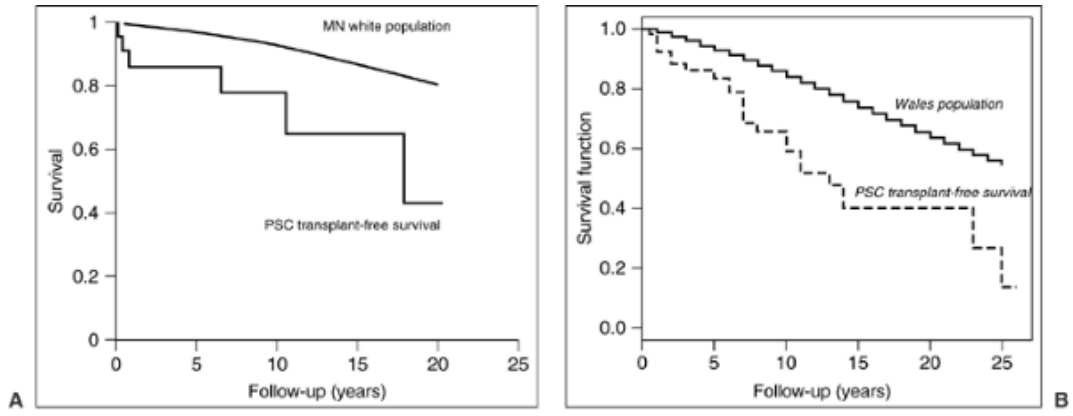
Choledocholithiasis Congenital abnormalities of biliary tract Caustic sclerosing cholangitis Ischemic stricturing of bile ducts Toxicity or stricturing of bile ducts related to intra-arterial infusion of floxuridine

AIDS, acquired immunodeficiency syndrome, PSC, primary sclerosing cholangitis.

Natural History

PSC is usually a progressive disease. In a retrospective study with 174 patients with PSC from the United States, the median survival rate from the time of diagnosis was approximately 12 years, which was less than that for an age-matched population (53). In another study from Norway, investigators estimated the median survival time of patients with PSC to be 17 years (51,54).

Recent population-based epidemiologic data also reported that PSC is a progressive disease and shortens life expectancy. Indeed, studies from Olmsted county, Minnesota, USA and Swansea county, Wales, UK that the liver transplantation free-survival was 65% at 10 years after the diagnosis of PSC and significantly less than the age- and sex-matched populations (Fig. 23.4) (3,4) This information further suggests that PSC is a progressive disease, and if suitable therapy were available, treatment early in the course of the disease would seem warranted.



▪ **Figure 23.4** Transplant-free survival of Olmsted, Minnesota, USA **(A)** and Swansea, Wales, UK **(B)** patients with primary sclerosing cholangitis compared with age- and sex-mathed population controls from the corresponding counties. PSC, primary sclerosing cholangitis. (Reprinted with permission from Bambha, et al. *Gastroenterology* 2003;125:1364–1369; and

Kingham, et al. *Gastroenterology* 2004;126:1929–30.)

Given the natural history studies, prognostic models have been developed to more accurately forecast an individual patient's disease progression, and thereby, define the best timing for liver transplantation. Using the Cox multivariable regression analysis the variables for these prognostic models were detected (48,50,51,52). To this end, the revised Mayo PSC natural history model employs five independent and reproducible parameters (i.e., age, total bilirubin, albumin, aspartate aminotransferase, and history of variceal bleeding) to estimate the survival of patients (48). Recently, the Model of End-stage Liver Disease (MELD) score has been widely used to prioritize PSC patients with end-stage liver disease before undergoing liver transplantation (55).

The natural history of patients with *small-duct* PSC is usually better compared to those suffering from classic PSC (56). Nevertheless, in some patients with *small-duct* PSC the disease can progress to classic PSC with typical cholangiographic features if these cases are followed up for several years (56). Finally, secondary sclerosing cholangitis has worse outcome compared to PSC (57). Finally, PSC predisposes to development of colon cancer and cholangiocarcinoma (see subsequent text). Indeed, patients with PSC and CUC have a significantly greater risk of developing colon cancer compared with patients having CUC only (58). Therefore, patients with PSC should undergo surveillance colonoscopies annually.

Associated Diseases

A variety of diseases aside from IBD have been associated with PSC (Table 23.2). It is unclear however, whether these associations are true, and therefore share a pathogenetic mechanism, or occur by chance and represent the reporting of two uncommon diseases.

The close association of PSC with IBD, particularly CUC, is now widely recognized. This association is found in 70% to 80% of patients with PSC. Crohn's disease has been reported as the cause of colitis in 10% to 15% of patients with PSC (14,59). However, Crohn's disease involving only the small intestine has not yet been described in patients with PSC. There is no clear temporal association between the diagnosis of PSC and IBD, although in general, the diagnosis of the latter is usually established before the liver disease is clinically apparent. Nevertheless, there are well-documented cases of IBD occurring years after the diagnosis of PSC. Similarly, patients can acquire PSC many years after proctocolectomy for colitis (21). In patients with PSC and colitis, the bowel disorder is one of relatively quiescent disease. However, the risk of development of colon cancer in patients with coexisting PSC and CUC seems to be substantially greater than if patients did not have PSC. There has been no association between the severity of bowel disease and the severity of liver disease, and therapy for the bowel disease does not affect the liver disease. Moreover, the most aggressive therapy for the bowel disease, proctocolectomy, has had no effect on PSC (21). Finally, there are no convincing differences in the liver disease of PSC patients with IBD compared to those without the latter.

Management

The complications of PSC can be due to (i) advanced-stage liver disease (i.e., portal hypertension, decompensated cirrhosis, and hepatic failure); (ii) chronic cholestasis; (iii) the underlying disease (i.e., specific for PSC). The management of complications of portal hypertension, decompensated cirrhosis, and hepatic failure is discussed in Chapters 11, 12, and 33. The management of complications related to chronic cholestasis and those specific for PSC are discussed in subsequent text. Chronic cholestasis can lead to pruritus, fat-soluble vitamin deficiency, metabolic bone disease, and steatorrhea. Specific PSC complications include gallstones and choledocholithiasis, dominant biliary strictures, cholangiocarcinoma and peristomal varices.

Table 23.5. Medications Used for the Management of Pruritus

Medication	Dosage
Ursodeoxycholic acid	15–30 mg/kg/d orally
Cholestyramine	4 g three to four times/d orally
Naltrexone	50 mg/d orally
Rifampin	150 to 300 mg two times/d

Management of Complications of Chronic Cholestasis

Pruritus

Although not common, pruritus can be disabling and associated with a diminished quality of life. The pathogenesis of pruritus in cholestasis is unknown, although endogenous opioids and retention of substances usually excreted in the bile have been considered (60,61,62). The intensity of the pruritus does not seem to parallel the severity of the liver disease, and pruritus may diminish as the disease progresses. Ursodeoxycholic acid (UDCA), cholestyramine, antihistamines and rifampin as well as opiate receptor antagonists have been used to treat patients with cholestatic pruritus (63,64). The usual doses of these medications are shown in Table 23.5. It is important to remember that rifampin, which can relieve pruritus in 3 to 5 days, can be associated with a reversible hepatotoxicity in approximately 15% of cases. Therefore, it is important to monitor the liver tests closely if this drug is used.

Fat-soluble vitamin deficiency

Fat-soluble vitamin deficiency is relatively common among patients with PSC, particularly as the patient progresses toward liver transplantation (65). As many as 40% of patients in some series have vitamin A deficiency; vitamin D and vitamin E deficiencies have been found among 14% and 2% of patients respectively (65). Vitamin K deficiency is uncommon. If this condition is suspected, a short trial of 10 mg water-soluble vitamin K can be considered. If

the prothrombin time responds after a few doses, long-term therapy should be recommended. Vitamin E deficiency is rare and unfortunately once established can be very difficult to correct with replacement therapy. The usual fat-soluble doses of vitamin replacement therapy are shown in Table 23.6.

Metabolic bone disease

Metabolic bone disease, usually caused by osteoporosis rather than osteomalacia, is relatively common and an important complication among patients with PSC (66). Glucocorticoids used to treat accompanying IBD aggravate the osteoporosis. Unfortunately, there is no proven therapy that can help these patients. Use of estrogen

replacement therapy by women should be considered. Calcitonin does not appear to be useful to patients with osteoporosis associated with PBC but it has not been tested in the treatment of patients with PSC. Bisphosphonates have been used with varying results to treat patients with PBC but have not been tested in PSC (67,68). Patients with PSC and IBD undergoing long-term glucocorticoid therapy for the latter, may benefit from oral administration of vitamin D and calcium, particularly those with bone density in the range of osteopenia (T score <1) or osteoporosis (T score <2.5).

Table 23.6. Vitamin Replacement Therapy for Primary Sclerosing Cholangitis

Vitamin A	25–50,000 units two to three times per wk, orally
Vitamin D	25–50,000 units two to three times per wk, orally
Vitamin E	100 units/d, orally
Vitamin K	5–10 mg/d, orally

Steatorrhea

Steatorrhea can occur in patients with PSC owing to diminished delivery of bile acids to the intestine either from coexisting chronic pancreatitis or celiac disease. All of these conditions should be considered in the evaluation of patients with steatorrhea in the setting of PSC (69) before treatment is recommended. If steatorrhea in patients with PSC is primary, low fat diet and substitution of medium-chain triglycerides for long-chain ones can improve the steatorrhea.

Management of Specific PSC Complications

Specific complications of PSC include cholelithiasis, choledocholithiasis, dominant biliary stricture along with recurrent cholangitis, cholangiocarcinoma, and peristomal varices in patients with an ileostomy after colectomy for colitis.

Gallstones and choledocholithiasis

Stones involving the gallbladder or bile ducts (i.e., choledocholithiasis) occur in approximately one third of patients with PSC. Chronic cholestasis predisposes to the formation of cholesterol gallstones and bile stasis with bacterial cholangitis leads to the formation of pigment stones of the bile ducts. Patients with PSC and choledocholithiasis may present with acute deterioration of liver tests if the bile duct becomes obstructed. This possibility should be considered if a patient with PSC has evidence of rapidly developing jaundice or bacterial cholangitis. Choledocholithiasis frequently can be treated endoscopically once the diagnosis is made.

Biliary strictures

In the largest series reported to date, dominant biliary stricture(s) occurred in only 7% of patients whose cases were followed up for up to 10 years. These strictures usually are in the extrahepatic biliary system and may be associated with jaundice, pruritus or relapsing bacterial cholangitis. If any of these symptoms occurs in patients with PSC, cholangiography should be considered. Evidence of dominant stricture(s) requires brush cytologic specimens for standard cytology studies as well as fluorescence in situ hybridization (FISH) and digitized image analysis (DIA) to exclude cholangiocarcinoma (see subsequent text). Often, bile duct strictures can be dilated endoscopically with a balloon catheter. Some authors have suggested that short-term biliary stenting is of value in improving the prognosis of liver disease, whereas others have shown that patients with biliary stents are at increased risk of complications (70). Direct surgical intervention for biliary strictures is seldom used and may predispose patients to recurrent bacterial cholangitis, because of the widely patent surgical anastomosis, and make future liver transplantation more technically demanding.

Prophylaxis for bacterial cholangitis should be considered in the care of patients with PSC undergoing biliary manipulation such as diagnostic or therapeutic ERCP. Ciprofloxacin or other broad-spectrum antibiotics are frequently administered both immediately before and for a short period of 1 or 2 days after the procedure because of the high biliary excretion.

Cholangiocarcinoma

Cholangiocarcinoma (CCA) is the most feared complication among patients with PSC. CCA may occur in 7% to 15% of patients with PSC at an annual incidence of 0.5% to 1% (71,72,73,74). Of interest, development of CCA does not correlate with severity of PSC. Chronic inflammation of bile ducts and cholestasis are likely predisposing factors associated with the development of CCA in PSC. Smoking and the presence of IBD have been suggested as predisposing conditions although they remain controversial. The diagnosis of CCA in the patient with PSC can be difficult to establish. The patient may have dominant biliary stricture(s), which is not easy to differentiate between benign and malignant lesion (i.e., CCA).

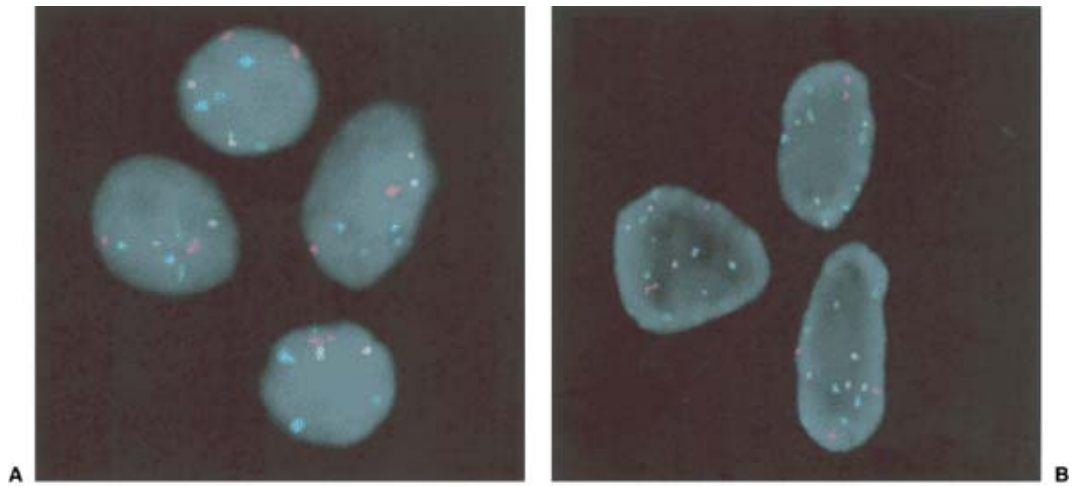
Serum markers for early detection of CCA in PSC have not been helpful. As a screening test, CA 19-9 does not appear to be sensitive enough, and the specificity is limited. For instance, CA 19-9 levels may be elevated due to bacterial cholangitis secondary to benign

strictures therefore limiting the specificity of the test (75,76,77). Nevertheless,

in a patient with PSC, sudden and unexpected clinical deterioration, which is associated with progressive elevation of alkaline phosphatase and serum CA 19-9 (>100 U/mL), in the absence of bacterial cholangitis indicates probable development of CCA.

New diagnostic methods such as DIA and FISH offer promise to evaluate bile duct lesions for cellular aneuploidy and chromosomal aberrations (77). To perform DIA and FISH assays, bile duct brushings are collected at the time of ERCP. DIA allows deoxyribonucleic acid (DNA) content quantificaion, assessment of chromatin distribution and nuclear morphology. In a recent study, DIA was compared to routine brush cytology for cancer detection in suspicious bile duct strictures (78). These authors reported that DIA was significantly more sensitive (39.3%) than standard cytology (17.9%, P = 0.014). Moreover, the accuracy of DIA was comparable to cytology (56% vs. 53%) (78).

The biliary FISH assay uses fluorescently labeled DNA-based probes to detect chromosomal aberrations in brushings of bile duct cells. A FISH assay is declared positive when five or more cells display gains of two or more chromosomes, or ten or more cells demonstrate a gain of a single chromosome (Fig. 23.5) (77). A recent study compared FISH to standard cytology for detection of malignant bile duct strictures. In this study, the sensitivity of FISH and cytology were 34% and 15%, respectively (P < 0.01). Additionally, the specificity of FISH and cytology was 91% and 98% (P = 0.06), respectively (79). Therefore, biliary FISH is more sensitive and virtually as specific as standard cytology for detection of malignant bile duct strictures (79).



▪ **Figure 23.5** Fluorescence in situ hybridization of normal **(A)** and maligant **(B)** bile duct cells. Normal cells display duplicate color for each chromosomal probe whereas malignant cells reveal multiple gains of chromosomes (i.e., polysomy). (Reprinted with permission from Lazaridis, et al. *Gastroenterology* 2005;218:1655–1667.)

Overall, in a patient with PSC, the diagnosis of CCA is usually made by combining the clinical exam, biochemical results, and imaging procedures (i.e., ERCP, MRCP). In medical practice, it is not uncommon to make the diagnosis of CCA based on clinical grounds and imaging studies without tissue proven evidence of tumor. To this end, physicians who evaluate and follow-up patients with PSC

should suspect development of CCA when these patients present with sudden clinical deterioration. Because we lack methods of early CCA detection in PSC the outcome for these patients is poor. Nevertheless, early diagnosis of CCA in PSC can be treated by liver transplantation in selected medical centers (80).

Peristomal varices

Peristomal varices can occur in patients with ileostomy after proctocolectomy for underlying IBD (81). The bleeding of peristomal varices can be severe and refractory to local measures, including ileostomy revision and injection of sclerosants. A transjugular intrahepatic portosystemic shunt or portacaval shunt can be considered, although many of these patients with bleeding peristomal varices have severe liver disease and portal hypertension and therefore, they should be considered for liver transplantation.

Management of PSC

Medical therapy

A variety of drugs have been tested as therapeutic agents for PSC (Table 23.7), but so far none has been found useful to treat the underlying disease. Penicillamine was the first drug tested on patients with PSC in a randomized trial but proved ineffective (82). Colchicine, methotrexate, and UDCA (13 to 15 mg/kg per day) have been unsuccessful to treat or delay PSC progression in randomized controlled trials (83,84,85,86). Other drugs that have been tested in small-scale studies, sometimes in open-label trials, have included nicotine, pirfenidone, pentoxifylline, and budesonide (20,87,88,89,90). The combination of colchicine and glucocorticoids led to some transient biochemical improvement (91). The combination of UDCA and methotrexate was not successful in an open-label trial (92). UDCA in higher doses (20 to 30 mg/kg per day) has appeared most promising in pilot studies (93,94) and large-scale randomized trials are being undertaken. At present, although high-dose UDCA seems promising, we do not recommend this agent in PSC patients except within the context of therapeutic trials. Of interest, UDCA has been shown to decrease the prevalence of colonic dysplasia in patients with PSC and CUC (95). Nevertheless, we need prospective studies to confirm the chemopreventive effect of UDCA in patients with coexisting PSC and CUC before implementation of prophylactic therapy.

Surgical therapy

Surgical therapy other than liver transplantation is seldom warranted for PSC. Biliary reconstruction has been suggested (96) but this aggressive approach has not been validated in prospective controlled studies.

Liver transplantation

The most pressing need of patients with PSC is for effective medical therapy for the underlying disease. Until this therapy is found, liver transplantation is the only option for patients with advanced PSC. The results of liver transplantation on patients with PSC have steadily improved; the 1- and 5-year survival rates are now reported to be 90% to 97% and 85% to 88% respectively (97,98). Nevertheless, PSC can recur after liver transplantation and as the follow-up period lengthens, the risk of recurrence seems to increase, although, the recurrent disease has been mild (99,100). Patients with PSC and CUC who have

undergone liver transplantation seem to be at high-risk of colon cancer. These patients need screening with annual colonoscopy and surveillance biopsies (101).

Table 23.7. Medical Therapy for Primary Sclerosing Cholangitis Tested to Date

D-Penicillamine
Cyclosporine
Pentoxifylline
Nicotine
Colchicine
Methotrexate
Budesonide
Pirfenidone
Azathioprine
Ursodeoxycholic acid

Conclusions

PSC is a chronic cholestatic liver disease of unknown causation. The course of the disease is typically one of slow progression. Various survival models provide individualized prognostic estimates. A number of complications can occur and should be considered in the treatment of these patients, the most important of which is the development of CCA. Unfortunately, no medical therapy is currently available for the underlying liver disease. Liver transplantation is an effective, life-extending option for patients with advanced PSC.

Additional research is necessary to understand the pathogenesis of PSC in order to develop better therapies for this devastating disease.

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Chapter 24
Primary Biliary Cirrhosis

Stephen P. James

Key Concepts

- Primary biliary cirrhosis (PBC) is an uncommon condition found throughout the world that primarily affects middle-aged women.
- Most patients are identified in an asymptomatic phase, but gradually they develop symptoms of pruritus, fatigue, and symptoms of associated syndromes or end-stage liver disease.
- Typical laboratory findings are elevations of alkaline phosphatase levels, modest elevations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, total cholesterol level, and positive antimitochondrial antibody test results.
- The characteristic pathologic features of PBC are destruction and drop-out of intrahepatic bile ducts, chronic portal tract inflammation, cholestasis, and progressive fibrosis, cirrhosis, and portal hypertension.
- About one half of patients have associated autoimmune syndromes, most commonly sicca syndrome, thyroid disease, arthritis, and scleroderma or calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia (CREST) syndrome.
- Treatment options include ursodeoxycholic acid (UDCA), cholestyramine, replacement of fat-soluble vitamins, management of complications of portal hypertension, and liver transplantation for end-stage disease.

Primary biliary cirrhosis (PBC) is a chronic, progressive disease of unknown etiology characterized by necrosis of intrahepatic bile ducts that leads to chronic cholestasis, portal fibrosis, and cirrhosis. The disease is characterized by female predominance, characteristic antimitochondrial antibodies (AMAs), associated autoimmune syndromes, and chronic inflammatory histologic features that have led to its classification as an autoimmune syndrome. Although the first descriptions of PBC appeared in the 19th century (1), wider recognition of the disease did not occur until the publication of reviews in the 20th century that clarified the clinical characteristics of this distinct syndrome (2,3). Other important developments were the discovery of AMAs (4), which led not only to an important diagnostic test but also to research into the pathogenesis of PBC that continues to the present time. Another advance was the introduction of cholestyramine to treat intractable pruritus and, more recently, ursodeoxycholic acid (UDCA) treatment to improve the biochemical features and retard the progression of disease. PBC is now frequently recognized in asymptomatic

patients, who may have a slow and insidious disease course. Nonetheless, most patients ultimately develop symptoms and have progressive disease. For those who develop end-stage liver disease, liver transplantation is the only therapeutic option.

Epidemiology

PBC appears to occur throughout the world and in all races; however, there is uneven geographic distribution (Table 24.1). Studies of the epidemiology of PBC are hampered by methodological problems, including variation in case definition and ascertainment and absence of noninvasive markers of high sensitivity and specificity. The estimates of incidence and prevalence vary widely, from 2 to 24 per million and 19 to 240 per million population, respectively, with the highest incidence and prevalence reported in northern Europe (5,6,7,8), where the prevalence appears to be increasing, although the relative contributions of methodological differences, increased awareness, or a true increase in prevalence are uncertain. The median age of diagnosis is approximately 50 years, with a broad range of diagnosis from approximately 20 to 90 years, and 90% of patients are women. Within regions of high prevalence, clustering of cases has been observed, suggesting a contribution of environmental factors to the pathogenesis (7). Two case–control studies have searched for risk factors with inconsistent results: In the United Kingdom an association with smoking was found, while in the United States an association with non-PBC autoimmune disease, genitourinary tract infections, and previous tonsillectomy was identified (9,10).

Genetics

There are multiple lines of evidence indicating that genetic susceptibility plays a role in the pathogenesis of PBC (Table 24.1). This evidence includes striking female predominance, familial clustering, association with other autoimmune syndromes, and concordance in twin studies (11,12,13). The striking female predominance of PBC obviously links the pathogenesis of the disease to the genetic basis of sex determination, as for many other autoimmune diseases. However, the specific causal mechanisms are currently unclear. Among candidate mechanisms are direct effects of female hormones, immunologic differences between males and females, and increased monosomy X in women with PBC (12). A commonly recognized feature of diseases that are thought to have an autoimmune basis is that different autoimmune diseases tend to occur with increased frequency within families, which forms the basis for the hypothesis that the tendency to autoimmunity is based in part on shared genetic traits. Numerous animal studies in models of lupus erythematosus, for example, support the concept of multigenic pathogenesis of complex autoimmune diseases. PBC has long been associated with a variety of autoimmune syndromes both in individual patients and within families of patients with PBC (see subsequent text). One recent population-based study confirmed that autoimmune conditions are found in approximately one half of patients and that the prevalence of autoimmune disease in first-degree family members is 14% (14), consistent with the thesis that PBC shares common genetic susceptibility traits with other autoimmune conditions. Many case series and epidemiologic studies have noted the familial tendency of PBC, which although uncommon, appears to be increased compared to the general population (12). The estimates of the familial prevalence has ranged from approximately 1% to a high of 6.4%, with estimates in the largest studies being in the 1% to 2% range. These estimates are similar in different

ethnic backgrounds, including England, the United States, Sweden, Italy, and Japan. Therefore, the familial occurrence of PBC also supports a genetic basis, although, of course, environmental etiologies also cause familial clustering. One of the important traditional methods to evaluate the heritability of disease is to determine concordance rates in twins. In two older reports of single twin pairs, one was reported to be concordant and the other discordant. More recently, an international study evaluated 16 twin pairs with PBC, of whom 8 were confirmed to be monozygotic (13). The concordance rate was 0.63 in monozygotic twins and 0.0 in dizygotic twins. The concordance rates were potentially subject to bias because cases were collected from referral centers and the Internet, which differs from traditional population-based twin registry studies. Nonetheless, the high concordance rate for monozygotic twins suggests an important contribution of genetic susceptibility to PBC.

Table 24.1. Epidemiology and Genetics

Incidence	2–24/1,000,000
Prevalence	19–240/1,000,000
Median age (y) (range)	50 (20–90)
Female	90%
Environmental risk factors	Unknown
Associated autoimmune syndromes	50%
Familial clustering	1%–2%
Twin concordance rate	Monozygotic 0.63/Dizygotic 0.0
MHC class II	Weak, variable associations

MHC, major histocompatibility complex.

Because PBC is a relatively rare disease and familial cases are uncommon, genome-wide linkage studies to identify novel genetic regions have not been possible. Therefore, the search for specific genetic susceptibility loci in PBC has been limited to association studies involving specific candidate genes. As for other autoimmune conditions, the major histocompatibility complex (MHC) region has been the subject of the most intense study. To date, no significant associations have been identified with MHC class I polymorphisms.

Numerous studies have reported both increased risk and protection associated

with specific MHC class II alleles. Recent reviews of these studies have concluded that there are different MHC class II associations in different ethnic populations, and furthermore, the associations have been relatively weak compared to other autoimmune diseases (12). To date no unifying hypothesis has emerged to explain the role of MHC class II alleles, as it has for other diseases such as celiac disease that are strongly associated with specific MHC class II alleles. A relatively short list of other candidate genes has been examined in PBC, with variable and inconclusive results. Future research using genome-wide single nucleotide polymorphism (SNP) markers for association studies offers promise to identify clues to the genetic basis of PBC.

Symptoms

In the past, patients were frequently diagnosed with initial symptoms of fatigue, pruritus, and jaundice, and physical findings such as hepatomegaly. However, currently, most patients are diagnosed with PBC before the onset of any specific symptoms or physical findings at an early stage of disease (Table 24.2). The reasons for the shift to early, asymptomatic diagnosis include the widespread use of screening laboratory studies including serum alkaline phosphatase, increased physician awareness of the features of the disease, the ready availability of AMA tests, and, finally, recognition that the disease does in fact have a long, presymptomatic phase in which a specific diagnosis is readily accomplished (15,16). Most patients, if followed up long enough, eventually develop symptoms that are attributed to the disease.

In order of frequency, the most common symptoms of PBC are fatigue, pruritus, and jaundice. Occasionally, patients have right upper quadrant abdominal pain. Many other symptoms may be present, including that of associated syndromes, such as dry mouth and eyes, Raynaud's phenomenon, and arthralgias. Occasionally, patients are brought to attention because of xanthoma, xanthelasma, increasing skin pigmentation, or fracture. Much less commonly, the presenting symptoms may be due to decompensated liver disease, including ascites, edema, bleeding, or encephalopathy.

Table 24.2. Symptoms and Physical Findings

Symptoms
Asymptomatic (most common)
Fatigue
Pruritus
Symptoms of associated syndromes (e.g., sicca, arthritis, Raynaud's)
Physical findings
Hepatomegaly
Splenomegaly
Jaundice
Xanthoma
Increased skin pigmentation, excoriations
End stage: Ascites, edema, wasting, encephalopathy

Fatigue may have an insidious onset and is often the dominant symptom, significantly diminishing the quality of life of patients (17). It is not uncommon

for patients to be initially misdiagnosed with other conditions in which fatigue is prominent, including depression, chronic fatigue syndrome, fibromyalgia, or thyroid disease, which may be associated with PBC. The cause of fatigue in PBC is unclear, but it has been suggested to have a central etiology (18), although other causes might exist, such as mechanisms related to chronic inflammation. Pruritus is also a significant, sometimes debilitating symptom in PBC, occurring much earlier in the course of the disease than in other more common forms of liver disease, in which it is commonly associated with hepatic decompensation. As in other cholestatic liver diseases, the pathogenic mechanisms of pruritus are unknown (19).

Physical Findings

Early in the course of the disease, physical abnormalities are often completely absent. As the disease progresses, the most common physical findings are hepatomegaly, splenomegaly, excoriations, skin hyperpigmentation, jaundice, and xanthelasma (20). Late in the course of the disease, physical findings of decompensated liver disease may be present, including ascites, edema, palmar erythema, spider nevi, muscle wasting, and signs of encephalopathy. Uncommonly, patients have physical findings of associated syndromes such as xerophthalmia and xerostomia. Very rarely, patients have Kayser-Fleischer rings because of copper retention.

Routine Laboratory Tests

The routine laboratory abnormalities in PBC are typical of chronic cholestatic syndromes and chronic liver disease (Table 24.3). Almost invariably, the serum alkaline phosphatase and γ-glutamyltransferase levels are elevated, typically two times to two-and-a- half times the upper limit of normal at the time of diagnosis (21). Serum alkaline phosphatase levels often remain relatively stable and do not correlate with the stage or progression of disease. Rarely, patients have been

identified with normal alkaline phosphatase levels on the basis of positive serum AMAs and typical liver histologic abnormalities, presumably at a very early stage of disease (22). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are only modestly elevated, usually less than five times the upper limit of normal, and their levels also do not correlate with stage of disease. Serum bilirubin level is typically normal early in disease and rises with disease progression; therefore, serum bilirubin is a component of the prognostic indices for end-stage PBC (23). In common with other cholestatic syndromes, serum lipid levels are abnormal in PBC. Patients may have striking hypercholesterolemia that may correlate with the physical finding of xanthomas. However, there is a corresponding increase in high-density lipoprotein cholesterol, which may explain why patients with PBC seem to have only an average risk for cardiovascular complications (24). In addition, although low-density lipoprotein (LDL) levels are also increased, a subfraction, lipoprotein X, is present that may inhibit the atherogenic properties of LDL lipoproteins (25). Late in the course of disease serum cholesterol levels may decline along with other synthetic functions during end-stage liver failure, including a fall in serum albumin level and rise in prothrombin time, which may be exacerbated by malabsorption of fat-soluble vitamins such as vitamin K. Because of the overwhelming fatigue experienced by some patients, concern may arise because of anemia in patients with PBC, which is usually due to anemia of chronic disease. Rarely patients are identified with autoimmune Coombs'-positive hemolytic

anemia (26), which may confound the interpretation of serum bilirubin levels, or pernicious anemia (27). Thrombocytopenia, if present, is usually a manifestation of portal hypertension, although rare cases of idiopathic thrombocytopenic purpura are associated with PBC. PBC, like other cholestatic syndromes, is associated with copper retention, resulting in raised urinary copper and serum ceruloplasmin levels (28). Other laboratory abnormalities may be present as a result of associated autoimmune syndromes. Patients with PBC typically have elevated serum IgM, sometimes to striking levels (3), some of which may be monomeric rather than pentameric (29).

Table 24.3. Characteristic Laboratory Abnormalities

Alkaline phosphatase	2 to 2.5 times upper limit of normal
AST, ALT	<5 times upper limit of normal
Bilirubin	Normal in early phase, progressive rise late phase
Cholesterol, HDL cholesterol	Elevated
Serum IgM	Elevated
Antimitochondrial antibodies	Present titer >1:40 in 95% of patients
Other autoantibodies	ANA, RF, SMA, and others commonly found

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; IgM, immunoglobulin M; ANA, antinuclear antibodies; RF, rheumatoid factor; SMA, smooth muscle antibody.

Autoantibodies

The presence of autoantibodies, particularly AMAs, is a clinical hallmark and important diagnostic feature of PBC, first reported as a distinguishing characteristic compared to other forms of chronic cholestasis by Doniach et al. in 1965 (4). Since this time, extensive studies have refined diagnostic testing for AMAs, which are found in up to 95% of patients (30). Furthermore, AMAs have been a major theme in research on pathogenesis of PBC. Initial studies defined AMAs by indirect tissue immunofluorescence, a test still used for clinical diagnosis. Subsequent studies showed that serum autoantibodies reacted with trypsin-sensitive components of the inner mitochondrial membrane, the so-called M2 antigen, in a non–tissue specific manner with broad cross-reactivity to antigens present in many species, including both eukaryotes and prokaryotes such as *Escherichia coli* (31,32). Other mitochondrial antigens, arbitrarily

designated M1 to M9 (33), have been previously described as the target of PBC autoantibodies. Some, such as M1, represent anticardiolipin of syphilis and are unrelated to PBC. Others, such as M4, M8, and M9, were previously described as being PBC specific; however, the specificity of these antigens has been questioned and they are not routinely used for diagnosis (34).

Cloning and characterization of the M2 autoantigens recognized by PBC sera have led to the identification of four major components of a family of mitochondrial antigens that contain lipoic acid and are members of the 2-oxoacid dehydrogenase (2-OAD) multimeric enzyme complexes (35,36). These include pyruvate dehydrogenase complex (PDC), 2-oxoglutarate dehydrogenase complex (OGDC), and branched-chain 2-oxoacid dehydrogenase complex (BCOADC). All the members of this family play important roles in cellular metabolism. Approximately 95% of sera of patients with PBC reacts with both the PDC core dihydrolipoamide acetyl transferase (E2) structure and the E3-binding protein (E3BP) by immunoblot or enzyme-linked immunosorbent assay (ELISA), these two subunits being completely cross-reactive. PBC sera also react, at a much lower frequency, with other components of PDC, including the E1α and E1β subunits. Sera also react with the E2 subunits of OGDC and BCOADC with a frequency of 90% and 50%, respectively. Considerable information is available on the B-cell epitopes, and they all appear to be defined by conformational

structures contained within the lipoic acid–binding domains. AMAs block the enzymatic activity of 2-OAD complexes in vitro (37,38); however, it is not known whether PBC autoantibodies have in vivo activity against these enzymes.

Although serum IgM may be elevated to striking levels, AMAs are present in all three immunoglobulin classes, including secretory IgA, and no consistent clinical correlations have been reported with different immunoglobulin classes of reactivity.

Although there has been considerable progress in the definition of AMAs in PBC, the literature consistently contains patients who are negative for AMAs but have otherwise the same typical clinical, biochemical, and liver histologic features of the disease (39,40). Some patients are found to be positive for AMAs when more sensitive and specific tests available in research laboratories are used (41). Some studies have reported a high frequency of antinuclear antibodies (ANAs) in this group of patients and have referred to this condition as autoimmune cholangiopathy (39,42). However, because the clinical features of the two conditions overlap considerably (43), it is unclear whether these are separate conditions or variants of the same condition with differing autoantibody patterns. The views on these conditions are more than semantic: The absence of AMAs in patients with PBC-like syndromes has been used as an argument that AMAs are only an epiphenomenon, rather than pathogenic, but this argument would be dismissed if future research on "autoimmune cholangiopathy" identifies a different etiologic basis compared to AMA-positive PBC.

Other autoantibodies are frequently found in patients with PBC. Approximately 25% of patients have ANAs with a "nuclear rim" and "multiple nuclear dots," which correlate with autoantibodies recognizing gp210, nucleoporin 62, and nuclear body protein sp100. These ANAs are relatively specific for PBC (44). Other autoantibodies that have been reported in patients with PBC include those connected with associated autoimmune syndromes (see subsequent text) such as antithyroid autoantibodies, rheumatoid factor, and antibodies found in Sjögren's syndrome, as well as a long list of other antibodies of unknown significance,

including antilymphocytotoxic antibodies, anti–acetylcholine receptor antibodies, antiplatelet antibodies, antihistone antibodies, anticentromere antibodies, and antibodies against carbonic anhydrase II, α-enolase, lactoferrin, and smooth muscles.

For clinical testing, historically, AMA is detected by indirect immunofluorescence on rat tissue sections. Reactivity is detected by the appearance of a cytoplasmic granular pattern of the mitochondria. False-positive reactions may occur because of the presence of other serologic cross-reactions, such as those with anticardiolipin (45). In addition, AMAs may be detected by immunoblot of submitochondrial particle preparations such as antigens or by ELISA using recombinant antigens for PDC-E2, BCOADC-E2, and OADC-E2. It appears that immunoblot and ELISA methods have higher sensitivity and specificity for PBC than immunofluorescence tests. AMAs may be found in patients with other syndromes, particularly overlap syndromes and autoimmune hepatitis (AIH), but also occasionally in primary sclerosing cholangitis (PSC) and drug-induced liver disease.

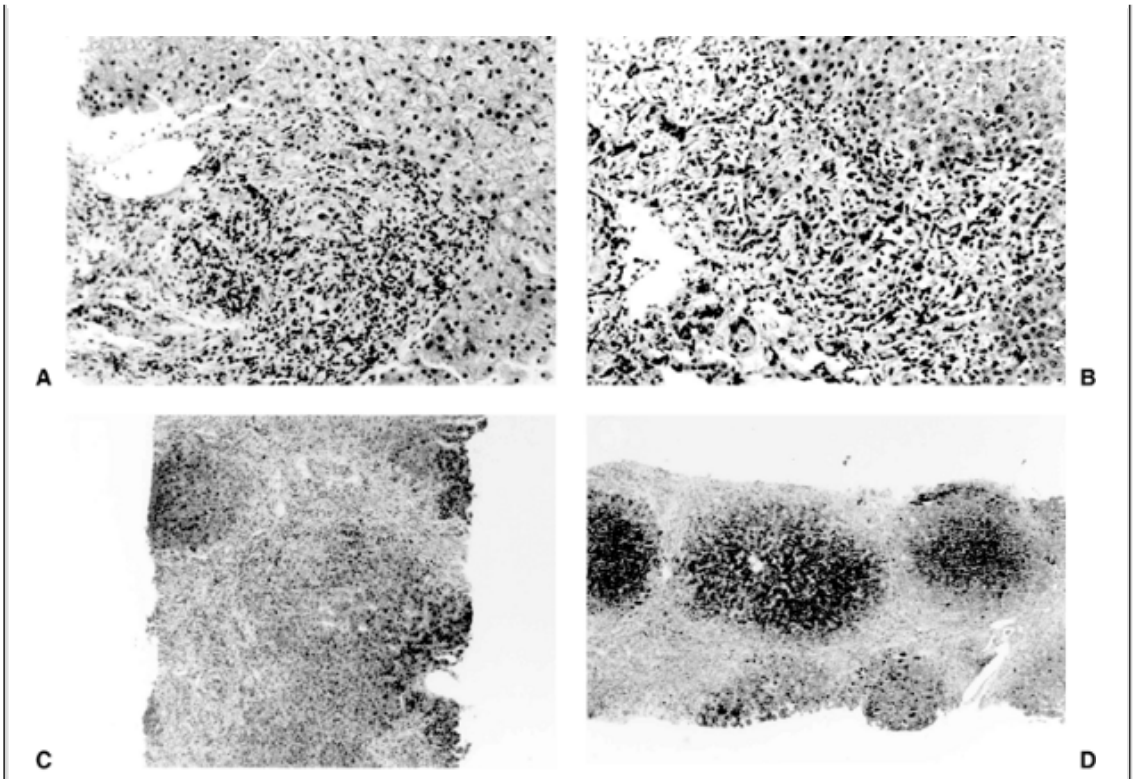
Pathology

The gross pathologic features of PBC are not specific for the disease and include bile staining of the liver, enlargement, fine nodularity, and, eventually, a grossly cirrhotic appearance. The extrahepatic biliary system is normal, with the exception of increased prevalence of gallstones. Enlarged lymph nodes may be found in the porta hepatis, along with other intra-abdominal sites, and these do not have any characteristic features other than the occasional appearance of granulomas. Enlargement of the spleen, along with other pathologic features of portal hypertension, appear later in the course of the disease.

The characteristic microscopic hepatic abnormalities include portal inflammation with destruction and disappearance of intrahepatic bile ducts, abnormal bile duct proliferation, fibrosis, and cirrhosis (Fig. 24.1). Although, overall, the disease is characterized by progressive destruction and loss of bile ducts with portal fibrosis progressing to cirrhosis, in the early and intermediate stages of the disease, the process is not uniformly distributed throughout the liver, and therefore, there is potential for significant sampling error when performing needle biopsy of the liver. Two similar staging systems, as proposed by Ludwig et al. (46) and Scheuer (47), have been widely adopted to describe the liver histologic lesions. Because of the focal nature of the lesions, occasionally multiple, and even all four histologic, stages of lesions have been identified in the same liver.

In stage 1, which is thought to represent the earliest pathologic lesions of the disease, there is destruction of intrahepatic septal and interlobular bile ducts, which can range in size up to 100 μm in diameter. This stage has been called the *portal stage* in the Ludwig system and the *florid duct lesion stage* in the Scheuer system. Portal lesions are characterized by damage to bile ducts, which may be associated with vacuolated or pyknotic biliary epithelial cells and even frank rupture or disappearance of portions of the duct. The duct is typically surrounded by a dense lymphocytic infiltrate, which may also include histiocytes, plasma cells, eosinophils, and, occasionally, true epithelioid giant cells. Neutrophils

are absent or infrequent, and periductular fibrosis, as found in PSC and biliary obstruction, is absent. Inflammation does not extend beyond the portal tracts, and hepatocyte injury is minimal.



▪ **Figure 24.1** Liver histologic abnormalities in PBC. **A:** Stage 1—a damaged bile duct is surrounded by chronic inflammatory cells. **B:** Stage 2—proliferation of abnormal bile ductules; chronic inflammation of portal tract. **C:** Stage 3—portal fibrosis. **D:** Stage 4—cirrhosis.

The second stage of the disease is characterized by the appearance of abnormal proliferating bile ductules without duct lumens, disappearance of normal bile ducts, and extension of the portal inflammation into the hepatic parenchyma. Typically, there is limited piecemeal necrosis of periportal hepatocytes with surrounding foamy macrophages and limited fibrosis confined to the portal tract.

In stage 3, there is a substantial increase in fibrosis, and fibrous septa may link portal tracts. Lymphocytic infiltrates remain in portal tracts, but bile ducts may be difficult to identify or completely absent.

Stage 4 is characterized by frank cirrhosis with regenerative nodules.

Other histologic abnormalities may be found that are not specific for PBC, including Mallory bodies and deposition of copper, which are found in other cholestatic liver diseases.

Immunologic techniques have been used to further define the histologic lesions of PBC (35,48,49). The lymphocytic portal infiltrates primarily contain $CD4^+$ T cells, although $CD8^+$ T cells predominate in areas of piecemeal necrosis, and their levels may be increased in early stages of the disease. The infiltrating $CD4^+$ T cells have a CD45RO high phenotype characteristic of memory T cells. The bile duct epithelium contains small numbers of intraepithelial lymphocytes, potentially analogous to those found in the intestinal epithelium, that are predominantly $CD8^+$ T cells and are found in PBC bile duct lesions. Natural killer T (NKT) cells represent only approximately 5% of lymphoid cells in liver infiltrates. Infiltrates

also contain B cells, plasma cells, eosinophils, macrophages, and dendritic cells. The cytokine profile of PBC tissue is predominantly characterized by expression of interferon-γ, but interleukin (IL)-2, IL-5, IL-6, and transforming growth factor-β (TGF-β) are also expressed, so that PBC does not have a clear T_H1 or T_H2 profile.

A number of bile duct abnormalities have been defined using immunologic techniques. Bile ducts in patients with PBC have aberrant expression of the AMA antigen PDC-E2 on the cell surface (50). In addition, MHC class II antigens, which are usually not expressed on normal bile ducts, have increased expression on bile ducts in PBC. Other molecules have also been noted to have increased expression on bile duct cells in PBC, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and the cytokines IL-6 and TNF-α (tumor necrosis factor-α), and the chemotactic molecule fractalkine (51).

Associated Syndromes

Numerous case reports and descriptions of patient cohorts have noted the association of PBC with other diseases, particularly autoimmune syndromes (Table 24.4). There are no extensive epidemiologic studies with case controls to evaluate the true prevalence of autoimmune syndromes in PBC, and many of the reports are likely subject to referral and reporting bias. In one study of a geographically based PBC cohort, the most commonly identified associated conditions were Sjögren's syndrome (25%); Raynaud's phenomenon (24%); autoimmune thyroid disease (23%); rheumatoid arthritis (17%); scleroderma and calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia (CREST) syndrome (8%); and pernicious anemia (4%) (52). The overall prevalence of autoimmune conditions in patients with PBC was 53% in this study. The association with Sjögren's syndrome, arthritis, Raynaud's phenomenon, and autoimmune thyroid disease is the most commonly reported associated syndrome in other reports on autoimmunity in PBC (53,54,55,56). The most common thyroid disease is Hashimoto's thyroiditis, but rarely Graves' disease or hyperthyroidism is found. Antithyroid antibodies are frequently found in patients with PBC without clinical evidence of thyroid dysfunction. Sicca symptoms are relatively common in PBC, but only a minority of patients have anti-Ro or anti-La antibodies typical of primary Sjögren's syndrome. Similarly, although arthralgia is common in PBC patients, only a minority fulfill American Rheumatism Association criteria for definite or probable rheumatoid arthritis. Patients having manifestations of scleroderma most often have the limited CREST variant of this disease. Among patients with PBC having one autoimmune condition, most also have a second condition.

Table 24.4. Associated Syndromes

Sjögren's syndrome[a]
Raynaud's phenomenon
Thyroid disease
Rheumatoid arthritis
Scleroderma and CREST
Systemic lupus erythematosus
Glomerulonephritis

Type 1 diabetes Polymyositis Myasthenia gravis Autoimmune thrombocytopenic purpura Pernicious anemia Addison's disease Celiac disease Skin diseases (e.g., lichen planus, pemphigoid, dermatomyositis)

[a]Listed in approximate order of decreasing frequency.
CREST, calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.

Overlap Syndromes

Patients with PBC may have features that overlap significantly with those of AIH (57,58). Positive AMAs, high serum alkaline phosphatase levels, and histologic bile duct lesions are sometimes found in patients with otherwise typical features of AIH. Conversely, patients with PBC may have significant hepatocyte injury and high–titer positive ANAs. Additional heterogeneity is represented by patients with typical clinical features of PBC except for the absence of AMAs, and some of these patients have high ANA levels. This latter syndrome has been called *autoimmune cholangiopathy* and is otherwise indistinguishable from PBC. There are no specific diagnostic criteria for overlap syndrome, so its prevalence in patients with PBC is unknown. Individual reports have indicated the presence of features of AIH in 2% to 19% of patients with PBC. Further, definition of the overlap syndrome will depend on new specific information on the etiopathogenesis of both conditions to differentiate whether overlap represents a spectrum of one disease or the simultaneous presence of two different conditions. The diagnosis of overlap syndrome is not purely academic because there are different treatment recommendations for AIH and PBC. In patients with significant features of AIH in addition to PBC, a treatment trial with corticosteroids and/or azathioprine should be entertained in addition to treatment with UDCA (59) (see subsequent text).

Complications

The early course of most patients with PBC who are not asymptomatic is marked by fatigue, pruritus, and, occasionally, symptoms or complications of associated conditions; however, patients often continue to have a good functional status. Sometimes, the symptoms of pruritus and fatigue may be quite disabling and emotionally disturbing to patients. Interestingly, depression does not seem to be a common diagnosis in PBC because there are alternative explanations for mood alterations. One small trial of an antidepressant in patients with fatigue found no benefit (60). Late in the course of the disease, the major complications are related to chronic cholestasis, portal hypertension, and cirrhosis (Table 24.5). Chronic cholestasis is associated with metabolic bone disease (61), which may cause painful fractures. The etiology of bone disease appears to be primarily the direct effects of cholestasis on bone, rather

than malabsorption of vitamin D. The full spectrum of complications of portal hypertension is found in patients with PBC, often before the appearance of stage IV cirrhosis on liver biopsy specimens (62). Historically, complications due to portal hypertension were common either as presenting symptoms or shortly after diagnosis, but these are now uncommon. There do not seem to be any unique features related to variceal bleeding, ascites, edema, encephalopathy, or wasting due to end-stage liver disease, with the possible exception of the additional contribution of malabsorption due to chronic cholestasis. Hyperlipidemia is a manifestation of chronic cholestasis, although the specific lipid abnormalities do not provide independent additional risk factors for cardiovascular disease. Although hepatocellular carcinoma has been reported in PBC, it may not be as common as other forms of cirrhosis (63,64). There have been reports of an increase in breast cancer in women with PBC, but this has not been confirmed by others. An increased frequency of asymptomatic urinary tract infection has been reported in PBC; however, the significance of this finding is unclear. Distal renal tubular acidosis has been reported in 30% to 60% of patients with PBC.

Table 24.5. Complications

Emotional disturbance: Debilitating pruritus and fatigue
Metabolic bone disease
Portal hypertension: Ascites, edema, encephalopathy, varices
Malabsorption: Fat-soluble vitamins
Hyperlipidemia
Hepatocellular carcinoma
Asymptomatic urinary tract infection
Distal renal tubular acidosis

Pathogenesis

The etiology and pathogenesis of PBC are unknown. A variety of etiologies have been postulated, including infectious causes; however, the most common view is that the disease falls within the category of autoimmune diseases (35). The rationale for this thesis is that multiple features of PBC support a primary autoimmune pathogenesis, including the histologic features of the liver bile duct lesions; characteristic autoantibodies; strong female predominance; an association, although weak, with MHC class II genes; the frequent association with other autoimmune syndromes; and the presence of similar bile duct lesions in other immunologically mediated diseases, namely, graft versus host disease (GVHD), both in humans and animal models, and the recurrence of PBC in liver allografts. One feature that unfortunately sets PBC apart from other autoimmune diseases is the apparent lack of benefit of immunosuppressive therapies. The lack of robust animal models for the disease has impaired rapid exploration of novel hypotheses, and patient-based research is hindered by the indolent and slowly progressive nature of the disease. Much current research into the pathogenesis of PBC parallels that of other autoimmune disease, including a search for environmental triggers, the genetic basis of susceptibility, and details about the

effector mechanisms responsible for the primary bile duct lesions that will hopefully lead to the identification of new therapeutic targets.

AMAs are the most distinctive specific immunologic characteristic of PBC and, not surprisingly, have been the focus of numerous studies. As noted in the preceding text, AMAs can inhibit the enzymatic activity of their target antigens in vitro; however, it is not known whether this occurs in vivo. Autoantibodies may potentially cause tissue injury, regardless of whether they alter the function of their target antigen; therefore, this particular issue does not resolve the question of the pathogenicity of AMAs. AMAs do fix complement; however, remarkably little attention has been directed at determining whether there is specific deposition of AMAs at sites of tissue injury in the liver. A number of attempts to induce biliary disease in animal models have met with limited success. In one study, immunization of mice with PDC-E2 resulted in an antibody response, however, without evidence of biliary disease (65). This result is not surprising because immunization with an autoantigen alone often does not lead to disease in animal models. In other model systems, the presence of multiple positive and negative genetic susceptibility genes is necessary in addition to environmental triggers to produce autoimmune disease (66); therefore, identification of a permissive genetic background is likely to be necessary to create animals with human disease, as has been demonstrated recently for dermatitis herpetiformis (67). One of the most intriguing pieces of evidence suggesting a potential pathogenic role for AMAs in liver disease is the association of neonatal hepatitis in two cases with the presence of AMAs of maternal origin, which resolved on disappearance of the AMAs (68).

Considerable information has emerged to define the nature of both the B-cell and T-cell responses to the autoantigens recognized by AMAs. There is overlap between epitopes recognized by B cells and both $CD4^+$ T cells and $CD8^+$ T cells, and there is enrichment for specific antigen-reactive clones within the liver (69). Cytokines produced in liver infiltrates are dominated by T_H1 cytokines but also contain T_H2 cytokines, providing a mixed picture. Furthermore, expression of adhesion molecules and chemokines, such as fractalkine, by biliary epithelial cells provide further details on potential mediators involved in the formation of inflammatory lesions within the liver. However, the precise sequence of events and specific effector mechanisms

contributing to bile duct injury are as yet undefined, and the possibility that the inflammatory lesions are entirely secondary in nature has not been eliminated.

Other animal models have been examined that might contribute insights into the pathogenesis of PBC. In an interesting example of serendipity, elimination of a specific diabetes-determining locus in the nonobese diabetic (NOD) mouse eliminates diabetes but results in a mouse with a characteristic spontaneous development of a unique liver phenotype consisting of peribiliary infiltrates, autoantibodies, and cystic biliary lesions (70). Another approach is based on the observation that patients with GVHD have histologic lesions of chronic nonsuppurative destructive cholangitis (71) that has features similar to those of PBC. This disease is mediated by the recognition of alloantigens by T cells, and the disease can be recapitulated in animal models, including the production of bile duct lesions (72). Although this model is useful in elucidating the specific immunologic mechanisms present in GVHD, it is as yet unknown whether the lessons learned extend to PBC.

The mechanisms that trigger the immunologic abnormalities observed in PBC

remain a matter of speculation. The highly conserved sequences in antigens are recognized by AMAs across species, including prokaryotes, suggesting that molecular mimicry could play a role in triggering autoimmune responses. Multiple microbes have been identified with sequence homology, including *E. coli*, *Neurospora crassa*, *Pseudomonas putida*, *Novosphingobium aromaticivorans*, and others (73). It has also suggested that xenobiotics might trigger immune recognition of self-antigens; however, no specific candidates have emerged in PBC.

Another theory for pathogenesis of PBC is that it is the direct result of an infection. In the search for a transmissible agent, filtrates of lymph nodes from patients with PBC were shown to induce the expression of PDC-E2 in biliary epithelial cell cultures (74). Further studies identified viral particles in biliary epithelial cultures and tissues from patients with PBC and cloning of exogenous retroviral sequences from cultured biliary cells, which were identified as a betaretrovirus related to murine mammary tumor virus (MMTV) (75). However, others have not been able to verify the presence of MMTV-related retrovirus in PBC (76). In an uncontrolled pilot study, combination therapy using lamivudine and zidovudine in a small number of patients showed improvement in liver histology and biochemical abnormalities, suggesting the possibility that antiviral therapy might be of benefit, but validation of this approach would require further controlled trials (77).

It is well accepted that PBC may recur (78) in approximately 20% to 30% of patients within 10 years of orthotopic liver transplantation, characterized by typical bile duct lesions and persistence of AMAs. Although recurrent PBC potentially affords an opportunity to study pathogenic mechanisms in the liver prospectively from the onset of disease, the mechanisms of recurrence per se are no less complicated to dissect than the primary disease itself, and as yet no new insights into pathogenesis have emerged from studies of recurrent PBC.

Diagnosis

The diagnosis of PBC is based on the presence of typical clinical features, routine laboratory tests, AMAs, liver biopsy specimen abnormalities, and absence of other conditions that could resemble PBC (20,79). PBC should be suspected in patients with symptoms such as profound fatigue, pruritus, abdominal pain, or hepatomegaly and routine laboratory test results consistent with cholestasis, that is, an elevated alkaline phosphatase levels with minimal or modest elevation of serum transaminase levels (less than five times the upper limit of normal). Other typical features are hypercholesterolemia and high serum IgM level. As noted in the preceding text, most patients are now diagnosed because of abnormal liver biochemical test results obtained on screening examinations in the absence of any symptoms. Occasionally, patients come to attention during the evaluation of symptoms of an associated syndrome, such as sicca syndrome, thyroid disease, or arthritis. The most important confirmatory laboratory test is serum AMA levels. The sensitivity and specificity of AMAs are both approximately 95%, but there is variation in the literature, depending on case definition and the test used (80). Approximately 5% of patients are AMA negative, and some of these patients have other autoantibodies, particularly ANAs. The latter patients are sometimes referred to as having autoimmune cholangitis; however, their clinical syndrome is not clearly distinguishable from PBC. Rarely, patients have been identified with normal serum alkaline phosphatase levels and positive AMAs, with typical liver biopsy findings.

Although, historically, liver biopsy was considered mandatory to make a specific diagnosis of PBC and provide prognostic information on the basis of histologic stage, a recent study has suggested that most patients do not need a liver biopsy for diagnosis (81). On the basis of a retrospective analysis of a large sample of patients who had undergone liver biopsy, AMA level determination, and serum biochemical tests, the combination of positive AMAs, alkaline phosphatase levels greater than 1.5 times the upper limit of normal, and AST less than five times the upper limit of normal (found in 112 of 131 patients; 85% of patients reviewed) had a positive predictive value of 98.2% for the diagnosis of PBC. Although other studies confirming this analysis have not been done, patients falling within this profile

probably do not need a liver biopsy unless other clinical features raise the possibility of a different diagnosis. Liver biopsy to confirm the diagnosis should certainly be entertained in the approximately 15% of patients who do not meet these criteria. An additional rationale for restricting the use of liver biopsy in patients suspected of having PBC is that valid prognostic information can be obtained from indices based on noninvasive testing, and histologic stage on liver biopsy is not needed for clinical decisions.

High-quality ultrasound is recommended as an initial diagnostic step in patients first presenting with a diagnosis of cholestasis, on the basis of routine biochemical studies. In the past, patients with PBC typically had endoscopic retrograde cholangiopancreatogram (ERCP) to rule out biliary obstruction or PSC; however, this is not indicated in those with typical clinical features of PBC, including high titer-positive AMAs, and in the absence of other findings that would raise suspicion for PSC, such as presence of inflammatory bowel disease (IBD). Patients with PBC have an increased prevalence of gallstones, but if there is no evidence of biliary obstruction, cholangiography is not indicated.

The differential diagnosis of cholestatic syndromes includes extrahepatic biliary obstruction due to stones, strictures, or tumors; PSC; drug-induced cholestasis; cholestatic viral hepatitis; sarcoidosis; idiopathic granulomatous hepatitis; AIH; and idiopathic adulthood ductopenia (Table 24.6). Other conditions that cause injury to bile ducts should be straightforward to differentiate and include GVHD, hepatic allograft rejection, ischemic cholangitis, and infectious cholangitis in immunosuppressed patients (82). Finally, clinical features of PBC may coexist with features of AIH in an overlap syndrome.

Extrahepatic biliary obstruction is diagnosed by imaging, and only rarely are patients found with positive AMAs. PSC should be suspected in patients with IBD. AMAs are only occasionally positive in patients with PSC. The diagnostic procedure of choice for PSC is cholangiography because the specific liver biopsy specimen lesions of PBC and PSC are each found in only a minority of their respective conditions, and nonspecific liver biopsy specimen features may be similar. Use of drugs known to cause chronic cholestasis could cause difficulty in diagnosis, particularly because some drugs also induce autoantibody production. Patients with viral hepatitis occasionally have a chronic cholestatic syndrome and bile duct lesions on liver biopsy specimens; however, AMAs are negative and serologic tests for viral hepatitis are positive. Occasionally, differentiation of PBC from hepatic sarcoidosis presents a difficult diagnostic problem (83). AMAs are typically negative in sarcoidosis, and the presence of typical extrahepatic manifestations is necessary to make the diagnosis of sarcoidosis. In about one fourth of patients with AIH, AMAs are positive, usually in low titer, and bile duct

lesions may be found on liver biopsy specimens. Idiopathic adulthood ductopenia is a somewhat recently described condition in adults characterized by paucity of bile ducts, chronic cholestasis, and, by definition, absence of any defining characteristics of other ductopenic diseases, including inflammatory liver histologic lesions, autoantibodies, or associated conditions such as IBD (84).

Table 24.6. Differential Diagnosis of Primary Biliary Cirrhosis

Extrahepatic biliary obstruction (e.g., stone, stricture, tumor)
Primary sclerosing cholangitis
Drug-induced cholestasis
Cholestatic viral hepatitis
Sarcoidosis
Idiopathic granulomatous hepatitis
Autoimmune hepatitis
Idiopathic adulthood ductopenia
Special situations with bile duct injury: Graft versus host disease, liver allograft rejection, ischemic cholangitis, infectious cholangitis in immunocompromised host

Treatment

Medical treatments of PBC have included primary therapies aimed at the underlying disease processes and preventing or delaying progression, therapies for symptoms such as fatigue and pruritus to improve quality of life, and therapies directed at complications, including osteoporosis, fat-soluble vitamin deficiency, and complications of portal hypertension, such as ascites, edema, variceal bleeding, and hepatic encephalopathy (Table 24.7). Comprehensive practice guidelines have been published, representing a consensus of expert opinion for management of PBC (85). There are no specific treatments for complications related to portal hypertension in PBC that differ from other causes of end-stage liver disease, and they will not be discussed here. Therapy may also be needed for associated syndromes, such as thyroid disease, arthritis, or Sjögren's syndrome, and they will also not be discussed here. In evaluating treatment trials for PBC, it should be kept in mind that this is a difficult disease to study because of many factors, including the indolent and variable natural history of the disease, sampling error in liver biopsy, and absence of robust biomarkers for early stages of the disease, in which therapeutic interventions might be expected to have the greatest benefit.

UDCA is the only U.S. Food and Drug Administration (FDA)-approved medical therapy for PBC, which is generally agreed to ameliorate serum

biochemical abnormalities and potentially delay progression of disease to death or need for transplantation, although controversy remains about the utility of the drug. A number of mechanisms have been proposed by which UDCA is therapeutic in PBC, including anticholestatic effects and antiapoptotic actions (86); however, the precise mechanisms are unclear and may not involve the primary abnormalities that lead to autoimmune destruction of bile ducts. Numerous treatment trials of PBC with UDCA alone, or in combination with other agents, have been conducted using varying numbers of patients and trial designs, and

these are summarized in detail elsewhere (87,88). In the largest reported trial, a Canadian multicenter double-blinded placebo-controlled study of 222 patients, treatment with UDCA at a dosage of 13 to 15 mg/kg per day resulted in improvement in transaminases, alkaline phosphatase, total cholesterol, and IgM levels and prevention of a rise in serum bilirubin level. However, no improvement in symptoms, liver biopsy results, or need for liver transplantation was noted. In a subsequent meta-analysis of 11 randomized trials involving 1,272 patients, improvement in biochemical studies was found, but no difference between placebo and UDCA was found for progression of liver histology or the need for liver transplantation (89). In contrast, a meta-analysis of the five studies (90,91,92,93,94) that included data obtained from open-label long-term follow-up periods reached a different conclusion, showing a significant reduction in risk of death or liver transplantation (odds ratio 0.68 [95% CI 0.48 to 0.95]) equivalent to a 32% reduction in patients treated with UDCA (95). Because UDCA consistently ameliorates serum biochemistry abnormalities, concern has been raised that predictive models for PBC might underestimate progression of disease; however, models such as the Mayo risk score still accurately predict the clinical course in patients with PBC who are receiving UDCA (96). Studies have also reported a delay in progression of liver fibrosis (97). A subsequent analysis of histologic data from four large placebo-controlled trials demonstrated that UDCA therapy had a beneficial effect in stage 1 or 2 disease, reducing periportal inflammation and ductular proliferation (98). Continuing analyses of open-label follow-up of patients have provided conflicting interpretations of the benefits of UDCA. In one randomized study in which patients received UDCA or placebo for 2 years, followed by open-label treatment in 112 patients for a subsequent 2 years, no differences were found in death or the need for liver transplantation (99). In another study of long-term UDCA treatment comparing treated and nontreated patients for a mean of 5.8 years, there was amelioration of serum biochemical abnormalities in treated patients; however, there was no significant difference in death, need for transplantation, symptoms, or complications. It should be noted that this study did not evolve from a randomized trial, and required data analyses taking into account baseline differences and prognostic models (100). Finally, another analysis of 262 treated patients followed up for a mean of 8 years, using a Markov model and the updated Mayo model for control predictions, found that treatment with UDCA normalizes survival rate (for death or transplantation) for patients with stage 1 or 2 disease who were treated, whereas those with more advanced stages had significantly worse survival. (101). Therefore, UDCA may be useful in earlier stages of the disease, although the search for more effective therapies needs to proceed.

Table 24.7. Treatment

Primary therapy	Ursodeoxycholic acid	13–15/mg/kg per day
Pruritus	Cholestyramine or colestipol Alternatives: Rifampin, phenobarbital, opioid antagonists, plasmapheresis	Up to 16 g/d in divided doses[a]

Metabolic bone disease	Calcium, vitamin D	
Malabsorption	Water-soluble forms of vitamins A, D, E, K	
Sicca	Artificial tears, dental hygiene	
Portal hypertension complications	As indicated	
Other associated syndromes	As indicated	

[a]May interfere with drug absorption; do not administer with other drugs.

Fortunately, UDCA is relatively well tolerated, the main side effect being diarrhea, and therapy appears to have no significant long-term complications. The currently recommended dosage is 13 to 15 mg/kg per day, although higher dosage regimens have been investigated. The cost-effectiveness of UDCA has been examined, with yearly costs estimated in 1998 to be $2,500 and annual cost-savings to be $1,372 on the basis of estimates of reduction in liver transplantation and development of esophageal varices (102). If cholestyramine is used concurrently to treat pruritus, there should be a 4-hour delay between administration of cholestyramine and UDCA. Multiple other agents have been tested as therapies for PBC, including azathioprine (103), methotrexate (104,105), prednisolone (106), chlorambucil (107), cyclosporin A (108), and

D-penicillamine (109). There is no clear overall benefit of most of these therapies, and some, such as systemic corticosteroids, are associated with significant bone complications (3). Although one prospective double-blind trial of colchicine showed improved survival after 4 years in one study, subsequent studies have shown much less benefit and no improvement in survival (20).

UDCA has also been used in combination with other drugs, including prednisolone, budesonide, azathioprine, methotrexate, and colchicine, drugs that individually have not shown consistent benefit for the disease in multiple trials. The results of these trials can be summarized as showing benefits no different from UDCA alone, and sometimes with more side effects (110). Small pilot studies have been undertaken with other agents, including mycophenolate mofetil, silymarin, and bezafibrate, but results of these studies are at best preliminary. At present, there is no clear evidence for any combination therapy using UDCA.

Pruritus is a distressing, and sometimes disabling, symptom of PBC. Although antihistamines are frequently used because they are well tolerated, most patients do not show an adequate response. UDCA treatment is associated with reduction

of this symptom in some patients, but overall results of randomized trials do not demonstrate significant improvement in response to pruritus. Cholestyramine has been the mainstay of therapy for decades (111). There are no randomized controlled trials of the anion exchange resins cholestyramine and colestipol; however, there is extensive clinical experience supporting their efficacy. Most patients have improvement in response to symptoms within about two weeks of initiating therapy. Typically, the drug is administered as 4-g doses, up to four times per day. These drugs are typically not well tolerated because of unpleasant taste, abdominal discomfort, and constipation. Anion exchange resins may contribute to malabsorption and may interfere with the absorption of other medications, including UDCA, thyroxin, digoxin, and oral contraceptives. Phenobarbital has been used occasionally and may be helpful. Rifampin has been used to treat pruritus at a dosage of 300 to 600 mg/day in divided doses (112,113). Although well tolerated, its use has occasionally been associated with hepatotoxicity and bone marrow aplasia. On the basis of the theory that pruritus occurs because of endogenous opioids, opioid antagonists have been used to treat PBC (114). Two randomized controlled trials of intravenous naloxone demonstrated benefit for intractable pruritus (115,116), and subsequent studies with oral naltrexone and oral nalmefene have also shown benefit (117,118). Symptoms of opioid withdrawal may occur as a side effect during initiation of opioid antagonist therapy. It has been suggested that the serotonin system may also be involved in pruritus (114), and drugs such as ondansetron and sertraline have been studied in patients with PBC; however, their potential benefit is as yet not substantiated by rigorous controlled trials. Plasmapheresis has been used as a therapy in intractable patients (119).

Metabolic bone disease, osteopenia, and osteoporosis may present a significant problem in patients with PBC. The pathogenesis is complex and may involve, among other factors, malabsorption of fat-soluble vitamins and, more importantly, direct effects of cholestasis on bone metabolism. Although their efficacy is uncertain, calcium and vitamin D supplementation are routinely provided for patients with PBC (120,121), calcium at 1.5 g/day and vitamin D at 800 IU/day. Two studies of etidronate provided mixed results on the benefits for patients with PBC (122,123); however, a more recent study with alendronate demonstrated favorable effects on bone mass (124). The role of hormone replacement therapy in women with PBC is unclear, given recent results in other conditions questioning efficacy in relation to the increased risk of cancer and vascular disease. Insufficient data are available to recommend calcitonin or sodium fluoride as therapy for bone disease in PBC.

Fat-soluble vitamin deficiencies may secondarily contribute to morbidity in PBC, including night blindness, bone disease, bleeding disorders or, rarely, neurologic symptoms of vitamin E deficiency. Deficiencies have been documented for vitamins A, D, K, and E, in decreasing order of frequency, with approximately one third of patients being deficient in vitamin A (125,126), with deficiencies correlating with advanced-stage disease and higher risk scores. Vitamin A supplementation should be based on serum retinol levels because excessive vitamin A may be hepatotoxic. Although the evidence base for the effectiveness of therapy is limited, it has been recommended that patients with hyperbilirubinemia be treated with water-soluble forms of A, D, E, K, and patients with prolonged prothrombin times can be treated with parenteral vitamin K with a 10 mg/month dosage (84).

Hyperlipidemia and xanthomas are common manifestations of PBC and

cholestasis, although patients with PBC may be relatively protected from the adverse effects of hyperlipidemia (24). Cholestyramine or colestipol (Colestid) is used to treat pruritus and UDCA because primary therapy may improve hypercholesterolemia. Nonetheless, additional therapy may be warranted in patients with other risk factors for cardiovascular disease. Clofibrate has been found to cause a paradoxical elevation of serum cholesterol level in two small studies (127,128). In one small study, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitor (statin) was shown to improve lipid levels without causing evident hepatotoxicity (129).

Other medical therapy considerations should include treatment for sicca syndrome, with artificial

tears, dental hygiene, antireflux, and vaginal lubricants and treatment of thyroid disease when present.

Liver Transplantation

Liver transplantation is the only option for patients with PBC with life-threatening end-stage liver disease and its complications or severe intractable symptoms. PBC is a relatively common indication for liver transplantation, representing 12% of patients in the European Liver Transplant Registry, which includes results for 3,357 patients with PBC undergoing transplantation between 1988 and 2003 (130). In general, patients with PBC are good candidates for transplantation and have a better long-term prognosis compared to those with other common indications for transplantation such as viral hepatitis or alcoholic liver disease, with 1-, 5-, and 10-year survival of 84%, 78%, and 69%, respectively (130); similar results have been obtained in the United States. Most experience is based on cadaveric transplantation, but living-related donor transplantation has also been performed successfully for PBC. The indications for liver transplantation in PBC include symptoms and signs of end-stage liver disease and its complications. In addition, patients have been considered for transplantation because of intractable severe pruritus, profound fatigue, and severe bone disease. Patients are typically considered for transplantation when their predicted survival is 1 year or less. The Mayo risk score has been validated as a reliable prognostic index for patients with PBC. More recently, the allocation of cadaveric organs in the United States is based on the Model for End-Stage Liver Disease (MELD) score (131). Both the Mayo risk score and the MELD score contain multiple factors, with serum bilirubin level being the major component of both, and a serum bilirubin level greater than 8.5 mg/dL generally correlates with Mayo and MELD scores that are indicative of a need for liver transplantation. PBC recurs in approximately 20% to 30% of patients by 10 years after orthotopic liver transplantation (78,132), characterized by typical bile duct lesions and persistence of AMAs. The diagnosis is based on liver biopsy and cannot be made on clinical features alone because of overlap with features of transplant rejection and drug toxicity. There has been interest in the possibility that transplantation immunosuppressive regimens may alter the natural history of the recurrent disease and, in particular, that some regimens may retard progression better than others. However, the recurrent disease does not appear to be of major clinical consequence in most patients, and there is too little information available to select transplantation regimens on the basis of disease recurrence (133). There have only been rare cases of retransplantation for recurrent PBC (78).

Natural History and Prognosis

The initial descriptions of PBC were based on observations in patients diagnosed at a relatively late symptomatic stage of disease, with relatively rapid progression to liver failure and death in approximately 6 years (2,3). The full clinical spectrum of PBC and its natural history are now much better defined. The disease has a variable spectrum but appears to be invariably progressive, whether presenting in asymptomatic or symptomatic patients. Multiple publications have indicated that overall survival to death or transplantation is better in asymptomatic patients, approximately 10 to 15 years (134,135,136), although significantly worse than in control populations. One large study of 770 patients found that the symptom status had little effect on overall mortality from all causes, with median survival of 9.6 versus 8.0 years in asymptomatic versus symptomatic patients, respectively (21). However, in the latter study, less than one third of deaths among asymptomatic patients with PBC were attributable to liver disease. In asymptomatic patients with a recent diagnosis (i.e., patients presumably in the early stages of the natural history of the disease), it is difficult to predict the ultimate course on the basis of serum biochemical or AMA titer, or liver histology. In considering the limited medical therapies currently available that may change the underlying disease (e.g., UDCA), management decisions do not depend on a precise definition of where the patient may be along the course of the natural history of the disease. Considerably more is known about defining the late stages of the natural history of the disease, which is important in considering the optimal timing for liver transplantation. Rising serum bilirubin level is an ominous prognostic sign in PBC (137). Multivariate analysis of survival in PBC as a function of multiple different parameters has resulted in the Mayo index, which has been validated in many subsequent analysis (138). More recently, the timing of transplantation has been supplanted by the use of the MELD score for prioritization for organ allocation. The MELD score is based on serum bilirubin level, creatinine level, international normalized ratio (INR) for prothrombin time, and etiology of liver disease, and it has been validated for prediction of death within 3 months for patients with PBC (139).

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Chapter 25

Postoperative Jaundice

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Key Concepts

- The causes of postoperative jaundice can be grouped under four major categories: Decompensation of preexisting chronic liver disease, cholestatic disorders, necroinflammatory liver diseases, and overproduction of bilirubin.
- A careful review of the surgical, anesthetic, transfusion, and pharmacologic treatment records, as well as history and physical examination are the initial steps in the evaluation of a patient with postoperative hepatic dysfunction.
- Additional laboratory tests and imaging studies are frequently necessary to exclude common liver disorders and biliary obstruction.
- These additional tests plus the information from the review of records and pattern and timing of liver biochemical abnormalities generally lead to the etiology of postoperative jaundice.
- Patients with chronic liver disease unrecognized before surgery are at risk of developing jaundice, and even liver failure, in the postoperative period.
- Postoperative jaundice caused by overproduction of bilirubin is characteristically associated with an isolated increase in unconjugated serum bilirubin in the setting of large volume transfusion, hematomas, or hemolytic disorders.
- Postoperative cholestasis (i.e., marked increase in alkaline phosphatase and bilirubin levels) should lead to suspicion of either biliary obstruction or entities associated with intrahepatic cholestasis.
- Hepatocellular necrosis caused by hepatic ischemia or acetaminophen and inflammatory disorders (viral and other

hepatitides) should be suspected when the postoperative hepatic dysfunction is expressed by prominent elevation of aminotransferase levels.

Patients who develop jaundice after a surgical procedure present a unique set of challenges to the clinician and require a careful assessment. Although in many instances the hepatobiliary abnormalities gradually resolve over time, often without specific intervention, in some patients the onset of postoperative jaundice may reflect a serious and potentially life-threatening complication. Because clinically significant liver injury can develop in the absence of jaundice, unless stated otherwise, the term *postoperative jaundice* is used in this chapter to also include abnormalities of other liver blood biochemical tests. There are often multiple factors present simultaneously in the postoperative patient that may lead to postoperative jaundice (1,2,3). The investigation of postoperative hepatic dysfunction is optimally accomplished as a joint enterprise by surgeons and physicians evaluating the patient's evolving clinical picture. This review presents a practical yet comprehensive approach to the diagnosis and management of a patient who develops postoperative jaundice.

Evaluation of Preoperative Risk Factors for Jaundice

The evaluation of a patient with postoperative jaundice begins with a careful review of preoperative and operative data, including operating room and anesthesia records. Key preoperative data to be analyzed include medical history, main symptoms, and physical examination performed before the surgical procedure. Preoperative clues for liver disease can be further investigated, if necessary, with the patient or family members. Questions on the existence of known chronic liver disease, use of therapeutic or illegal drugs, and a detailed history of alcohol consumption should be obtained. Preoperative diabetes mellitus, obesity, female gender, hyperlipidemia, and other features of the metabolic syndrome suggest that the patient may have underlying nonalcoholic fatty liver disease (NAFLD) or its more aggressive variant, nonalcoholic steatohepatitis (NASH) (4). Historical risk factors for acquisition of hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, such as a history of intravenous drug use or blood products transfused before 1992, should prompt the determination of HCV antibody and hepatitis B surface antigen (HBsAg) status. A history of recurrent mild jaundice based on unconjugated hyperbilirubinemia suggests Gilbert's syndrome. Although the postoperative patient with Gilbert's syndrome may develop more pronounced jaundice, the prognosis is excellent and no further investigations are necessary other

than documenting a predominantly indirect hyperbilirubinemia.

A review of preoperative physical examination records may reveal signs of the existence of underlying chronic liver disease, which perhaps went unrecognized preoperatively. Spider telangiectasis, gynecomastia, palmar erythema, Dupuytren's contractures, hepatomegaly, and/or splenomegaly would suggest preexisting chronic liver disease.

Evaluation of preoperative laboratory data can also be useful to assess for the presence of preexisting chronic liver disease. Elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels suggest chronic hepatitis, whereas the existence of preoperative thrombocytopenia (even mild), depressed serum albumin level, and/or prolonged prothrombin time suggest the presence of cirrhosis, which may have been otherwise well compensated before the surgical procedure. Similarly, preoperative imaging studies revealing hepatosplenomegaly and/or intra-abdominal varices are helpful in raising the suspicion of preexistent cirrhosis.

After major surgery, patients with cirrhosis have a distinct risk of decompensation (i.e., development of jaundice, ascites, hepatic encephalopathy, or portal hypertensive gastrointestinal bleeding). Furthermore, patients with cirrhosis are more susceptible to hepatic ischemia during intraoperative arterial hypotension or hypoxemia. This in turn can be expressed postoperatively as onset of abnormal liver biochemistries or even frank hepatic decompensation.

In general, the probability of postoperative decompensation and liver failure is related to the existence of residual hepatic reserve before the surgical procedure. This has been traditionally evaluated by determining the patient's Child-Turcotte-Pugh class (5,6). Child-Pugh class A patients are considered to have a probability of decompensation of 10% or less, whereas Child-Pugh class B or C carries postoperative mortality risks of 20% to 30% or 40% to 60%, respectively. Therefore, learning that the patient with postoperative jaundice had a diagnosis of cirrhosis made preoperatively can be important in the evaluation, management, and prognosis of hepatic dysfunction. More recently, the postoperative risk evaluated by the more objective Model for End-Stage Liver Disease (MELD) score was found to be superior to Child-Pugh method in predicting outcome (7,8).

When evaluating a patient with postoperative jaundice or abnormal liver biochemistries, it is reasonable to check the basic viral hepatitis serologies, iron indices, and antinuclear and smooth muscle autoantibodies and obtain a complete abdominal imaging study. If the etiology remains obscure and the abnormalities persist or worsen, additional investigations are warranted. It is important to note that in chronic infection with HCV, and also in other hepatic diseases, cirrhosis may be present in spite of normal aminotransferase levels (i.e., ALT,

AST).

Causes of Postoperative Jaundice

A large number of conditions may lead to postoperative hepatobiliary dysfunction (1,2,3). The main causes can be grouped into four main categories: Cholestatic problems including extrahepatic cholestasis (obstructive jaundice) and intrahepatic cholestasis, necroinflammatory liver diseases, excessive production of bilirubin, and preexisting hepatobiliary disease that becomes apparent postoperatively. The pattern and timing of the abnormalities can be helpful to narrow the differential diagnosis (Tables 25.1 and 25.2).

Postoperative Jaundice due to Biliary Obstruction

Major abdominal surgery can be associated with postoperative jaundice. Classic examples include open

aortic surgery, esophageal surgery, and other procedures involving or adjacent to the porta hepatis. In particular, the occurrence of acalculous cholecystitis after open cardiac or aortic surgery should be kept in mind. Insertion of hepatic arterial infusion pumps with administration of chemotherapy can also lead to cholecystitis, and even to secondary sclerosing cholangitis. Acute portal vein thrombosis is a rare but severe complication after major upper abdominal surgery or in patients with inflammatory bowel disease (9). Acute postoperative portal vein thrombosis can lead to liver failure if not promptly treated. Suspicion should be high in the setting of conditions associated with hypercoagulable states. Idiosyncratic forms of liver dysfunction have also been reported with large abdominal surgery (10). Surgery of the biliary tract may be followed by postoperative jaundice. Patients undergoing cholecystectomy for gallstones may have retained stones, or worse, a bile duct injury (11). Generally, patients present 3 to 4 days after surgery; although in a recent large study, the mean time to referral for bile duct injury was 3 weeks (12). Patients often present with severe right upper quadrant pain or even generalized peritonitis. Fever, nausea, and vomiting may also be present. Variable elevation of the total bilirubin, alkaline phosphatase, and aminotransferase levels can be present. Immediate evaluation with hepatobiliary ultrasonography and magnetic resonance cholangiopancreatography (MRCP) should be obtained to investigate biliary leak, biloma, or abscess. Hydroxy iminodiacetic acid (HIDA) biliary scan may be useful if MRCP is not readily available. Endoscopic retrograde cholangiopancreatography (ERCP) is often subsequently used for confirmation of the diagnosis and therapeutic intervention. If stones are present, removal with or without sphincterotomy is indicated (13,14). If a cystic or bile duct leak is found, placement of an internal

biliary stent is often helpful. In patients who present very late, a biliary stricture may be found. Balloon dilatation, with repeated biliary stent changes, is the first line of therapy (15). If this fails, or in more acute and severe injuries such as complete transection, surgical exploration with repair is necessary (16). In a recent review of 200 patients treated over 13 years for bile duct injury after laparoscopic cholecystectomy, 175 required some form of surgical intervention (12). Jaundice was present in 50%, whereas bile leak, cholangitis, biloma, and uncontrolled sepsis were evident in 59%, 35%, 5%, and 3%, respectively. Early referral to a tertiary care center with surgeons experienced in hepatobiliary surgery is important to minimize complications, number of required surgeries, and overall mortality. Immediate repair appears preferable but if diagnosis is delayed, control of sepsis and persistent bile leak should first be accomplished. Once peritonitis has resolved, biliary reconstruction can be scheduled after 2 to 8 weeks.

Specialized endoscopy and interventional radiology are helpful adjuncts for the successful care of these patients.

Table 25.1. Causes of Postoperative Obstructive Jaundice and Time of Presentation

Within 2 to 3 wk of surgery
Decompensation of preexisting chronic liver disease
Overproduction of bilirubin: Hematomas, transfusions, hemolysis
Hepatic ischemia: Vascular injury, cardiac failure, shock, bleeding, hypoxemia
Benign postoperative intrahepatic cholestasis
Biliary obstruction: Retained stones, bile duct injury or leak, pancreatitis
Therapeutic drug liver injury: Acetaminophen, other drugs
Gilbert's syndrome
After 2 to 3 wk of surgery
Therapeutic drug-induced liver injury
Total parenteral nutrition
Viral hepatitis

Modified from Baker A, Green R. Postoperative jaundice. In: Schiff E, Sorrell M, Maddrey W, Eds. *Diseases of the liver*. 9th Ed. Philadelphia: Lippincott Williams & Wilkins, 2003.

Table 25.2. Common Patterns of Liver Blood Test Abnormalities in Postoperative Jaundice

Mechanism of postoperative jaundice	Pattern
Bilirubin overproduction	Isolated increase in unconjugated bilirubin level, occasionally mild increase in AST, ALT levels
Hepatic ischemia	Mild to severe elevation of ALT, AST, and LDH levels; bilirubin level elevation and prothrombin time prolongation in severe cases; normal AP and albumin concentration
Viral and drug-induced hepatitis	Variable increase in the levels of bilirubin, ALT, AST, and AP; broad spectrum of abnormalities
Biliary obstruction	Moderate increase in level of bilirubin, marked increase in AP level, and mild to moderate increase in ALT and AST levels
Benign postoperative intrahepatic cholestasis	Mild to marked increase in bilirubin and AP levels
Gilbert's syndrome	Mild increase in unconjugated bilirubin content

AST, aspartate amino transferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; AP, alkaline phosphatase.
Modified from Baker A, Green R. Postoperative jaundice. In: Schiff E, Sorrell M, Maddrey W, Eds. *Diseases of the liver*. 9th Ed. Philadelphia: Lippincott Williams & Wilkins, 2003.

Postoperative jaundice is predictable in patients undergoing biliary reconstruction for benign or malignant disease. Swelling at anastomotic sites is thought to be responsible, and normalization of liver blood test results may take several days. If laboratory test results do not resolve, bile leak or stricture must be ruled out.

Hepatic resections can lead to postoperative jaundice, although usually of transient duration. However, if there is underlying liver disease, or a substantial portion of normal liver was resected, patients may have more severe, even progressive, liver dysfunction. The course of this form of hepatic failure usually extends over days to weeks, with sepsis frequently responsible for death. If not contraindicated, liver transplantation may be necessary. When an extensive hepatic resection is anticipated, preoperative techniques such as selective portal vein embolization or ligation may be performed to induce growth of the lobe that is free of disease (17,18).

Rarely, postoperative jaundice can be seen in patients who develop pancreatitis after surgery. Often, this is associated with large procedures such as open cardiac or aortic cases (19,20). However, pancreatitis can develop after gastric or splenic procedures as well. Edema of the pancreatic head with external compression of the bile duct probably plays a role in the pathogenesis. Liver blood biochemical test results gradually resolve as the pancreatitis subsides.

Postoperative Intrahepatic Cholestasis

A predominant abnormality of alkaline phosphatase and bilirubin levels in the absence of large duct obstruction clinically defines intrahepatic cholestasis in the postoperative patient. The patient may develop varying degrees of jaundice, dark urine, pruritus, and acholia. The prothrombin time may be prolonged because of vitamin K malabsorption associated with cholestasis. The pathophysiology underlying intrahepatic cholestasis involves processes that disturb bile flow at the microscopic level, either at the biliary membrane of the hepatocytes or at the level of the fine cholangiolar ductules (21). The nonspecific nature of histologic findings (i.e., canalicular bile plugs and bile staining) makes liver biopsy rarely useful in precisely identifying the cause of postoperative intrahepatic cholestasis. Liver biopsy, however, may be helpful in excluding the presence of underlying liver disease before the surgical procedure (see subsequent text).

The most important clinical aspect when diagnosing postoperative cholestasis is to clearly ascertain the absence of mechanical biliary obstruction. Once the patency of the macroscopic biliary tree has been established, the clinician must consider the various causes of intrahepatic cholestasis. A broad number of liver disorders can occasionally present with a cholestatic pattern, including therapeutic drug–induced hepatic injury, infections, and cholestatic variants of

viral hepatitis.

Therapeutic Drug–Induced Hepatic Injury

Several pharmacologic agents frequently utilized in the perioperative period have the potential to cause liver injury. These include excessive use of acetaminophen and idiosyncratic hepatotoxic reactions to many antibiotics, anticonvulsants, and cardiovascular agents. Some commonly used agents with potential for hepatotoxicity include sulfas, penicillin derivatives, nitrofurantoin, phenytoin, amiodarone, statins, metronidazole, and fluconazole. Although acetaminophen administered in therapeutic doses is not hepatotoxic, liver injury may develop with routine doses in patients who regularly consume alcohol and/or have endured prolonged fasting, which deplete glutathione reserves (22). These individuals may develop elevated postoperative aminotransferase levels as a result of inadvertent acetaminophen overdosing, or as a result of the preceding factors that enhance acetaminophen hepatotoxicity. A detailed review of drug-induced hepatotoxicity can be found elsewhere in this textbook (see Chapters 33 and 34). Because of the large number of drugs with the potential to cause postoperative hepatic dysfunction, a careful history and review of the records documenting the agents used before, during, and after the surgical procedure is a key element of the investigation of postoperative liver injury. In general, drugs that the patient had been taking for more than 1 year preoperatively are unlikely to be involved in postoperative hepatotoxicity. Drug-induced liver injury generally develops within a few weeks and up to 12 months of exposure to the offending agent (1,2,3). Consequently, very early postoperative jaundice or liver biochemical abnormalities are unlikely to be related to drugs (with the possible exception of acetaminophen overdose). In contrast, hepatic dysfunction developing 2 to 3 weeks after surgery, or after initiation of new pharmacologic therapy, should be considered suspicious of drug-induced liver injury. The biochemical and histologic patterns of drug-induced liver injury encompass the broad spectrum of hepatic pathology. The cause–effect relation between a suspected agent and postoperative liver damage should be ascertained by assessing the temporal sequence of events and excluding other types of postoperative liver dysfunction, such as hepatic ischemia, viral hepatitis, and the cholestatic disorders outlined earlier. Liver biopsy is occasionally necessary to exclude other

etiologies, but more often, there are no histologic features that are diagnostic of drug-induced liver disease.

Hepatitis and Cholestasis Associated with Anesthetic Agents

General anesthesia is a well-known cause of postoperative jaundice.

Careful review of the anesthetic record is important when determining the etiology of postoperative hepatic dysfunction. Certain anesthetic agents, sustained decrease in systolic blood pressure with subsequent reperfusion, and the need for cardiopulmonary bypass can all lead to severe liver injury (23,24,25).

The most common manifestation, however, is transient hepatic dysfunction resulting from a reduction in splanchnic arterial blood flow during general anesthesia (25). Often, this is not correlated to a dramatic change in systolic blood pressure on the anesthetic record. In patients with portal hypertension, intraoperative hemodynamic changes can be of greater consequence. In cirrhosis, there is frequently reduced portal venous flow, and the hepatic artery is the primary source of inflow to the liver (5).

In cases of true ischemic injury, aminotransferase levels can be elevated up to 100 times the normal value, along with significant elevations in lactate dehydrogenase (LDH) levels within the first few days. Alkaline phosphatase levels increase minimally (26). If the injury is severe, patients can further develop subfulminant or fulminant hepatic failure. In general, the liver blood biochemical test results return to normal within a week, although bilirubin may take longer to normalize. Liver biopsy can be useful to rule out underlying liver disease. Centrilobular (zone 3) hepatocellular necrosis is generally found and congestion is observed when cardiac failure has had a role in the ischemic injury. In general, a liver biopsy is not necessary to confirm the presence of postoperative ischemic hepatic injury (27,28).

Certain volatile anesthetic agents have been implicated in the development of hepatitis. The most well-known instance is that of halothane and its metabolites (29,30,31). Halothane-associated hepatitis is estimated to occur in 1 of 10,000 halothane doses (32). Although hepatitis has been reported to occur after a single administration of anesthetic, the more common history involves multiple exposures. As the number of exposures increases, the severity of the hepatitis worsens. Some lethal reports have involved exposure intervals of less than 3 months. Patients at risk are often obese, older than 30 years, and of female gender (1,33).

The two mechanisms thought to be involved are direct hepatic toxicity and the production of immunogenic protein complexes. All volatile anesthetic agents undergo some degree of biotransformation by the hepatic cytochrome P-450 system. The triacetyl chloride produced is the most troublesome because it binds covalently to hepatic macromolecules (34,35). Although this metabolite can lead to direct hepatocyte injury by itself, the more significant action of this protein immunocomplex is its initiation of the inflammatory response. Activation of the inflammatory cascade leads to amplification of the reaction and sensitization (36). Therefore, there is more severe

reaction with each added exposure.

Although elevation of liver blood biochemistries can be seen early after the exposure, symptoms can manifest as late as 2 to 3 weeks after administration. With each subsequent exposure, symptoms may present earlier. Patients can develop fever, nausea, vomiting, and rash followed by jaundice. They may also complain of arthralgias and right upper quadrant pain and have hepatomegaly on physical examination. Laboratory evaluation reveals significant elevation of liver biochemical test results depending on the severity. In mild to moderate cases, aminotransferase levels will be slightly elevated. However, in severe episodes, aminotransferase levels can be up to ten times the normal value, with significant elevation of LDH and serum bilirubin levels and decrease in concentration of coagulation factors. Usually, the liver function returns to normal within the first few weeks. However, progressive liver failure can occur, and mortality rates of up to 60% have been reported (1). Histology varies among reports (29,37). In moderate cases, pathology reveals centrilobular necrosis, with intense mononuclear infiltrate. In more severe cases, massive hepatic necrosis can be observed. Often, there is eosinophilia, supporting a role for an immune response. Although these changes are usually transient, chronic hepatitis with piecemeal necrosis has been seen on liver biopsy specimens as late as 10 months after exposure (38).

Other halogenated agents used for anesthesia include enflurane, methoxyflurane, sevoflurane, isoflurane, and desflurane. Of these, enflurane and methoxyflurane have been more frequently reported to cause drug-induced hepatitis (39). The likelihood of developing a reaction is associated with the rate of biotransformation of each drug. Halothane, with a rate of 20%, is more likely to cause hepatitis than enflurane, sevoflurane, isoflurane, or desflurane, with rates of 2.5%, 1%, 0.2%, and 0.02%, respectively (35). Scattered reports do exist in the literature for each of these agents, although they remain quite rare. Because there is cross-reactivity among these agents, exposure to any of them may lead to reactions to the rest. For instance, all the reports involving desflurane or isoflurane document prior exposure to halothane or enflurane.

Treatment consists of withdrawal of the causative agent and supportive care. If hepatic failure develops, urgent liver transplantation may be required. Once a

patient has had documented liver dysfunction from any of the halogenated anesthetics, it is recommended that their use be avoided on subsequent surgical procedures.

Postoperative Cholestasis Due to Infection

Postoperative sepsis can be accompanied by abnormal liver biochemical

test results. The total bilirubin level, including an increase in direct-reacting bilirubin, is often elevated to a range between a few milligrams per deciliter and sometimes greater than 20 mg/dL. Alkaline phosphatase and aminotransferase levels may also be abnormal, but generally to a lesser degree than those of bilirubin and in a nonspecific manner. Infections with anaerobes, aerobes, and gram-positive and gram-negative bacteria can lead to postoperative intrahepatic cholestasis. Pyelonephritis or urinary tract infection, appendicitis, cholecystitis, diverticulitis, or peritonitis in the postoperative patient can be associated with the development of cholestatic reactions. Infection-related cholestasis should be suspected in the presence of fever, leukocytosis, positive blood cultures, or evidence indicating an active infectious process (e.g., abscess on imaging studies and/or purulent drainages). The diagnosis of infection-related hepatic dysfunction should also be considered when the etiology of postoperative jaundice remains obscure and other causes of jaundice have been appropriately ruled out. Liver biopsy is not required for the diagnosis because of the lack of specific histopathologic features. If performed, however, hepatic histology often reveals ductular proliferation, polymorphonuclear-based cholangitis, and canalicular cholestasis. The management of postoperative jaundice associated with infection should focus on the treatment and control of the specific infection.

Viral Hepatitis

Currently, transfusion of blood products carries a very small risk of transmitting hepatitis B or C viruses (40). Consequently, acute viral hepatitis has become a rare cause of postoperative jaundice. Postoperative acute viral hepatitis is likely to have been acquired before the surgical procedure, and without knowing this the patient may have undergone the surgery during the incubation period of the hepatitis virus. The elevation of ALT and AST levels may be detected at any time after surgery, depending on the timing of the infection and the virus incubation period. The characteristic biochemical profile is that of a marked elevation of the content of aminotransferases, often to greater than ten times the upper normal level. In severe cases, bilirubin level also increases and coagulopathy may develop. The serologic diagnosis, management and prognosis of acute viral hepatitis A, B, or C, is reviewed in detail elsewhere in this textbook (see Chapter 26). Other viruses that may cause hepatitis (e.g., Epstein-Barr, cytomegalovirus, herpes simplex) are even less common in the postoperative setting, but they should be kept in mind when dealing with immunosuppressed patients (41).

Benign Postoperative Cholestasis

A frank cholestatic syndrome may develop postoperatively with

prominent elevation in alkaline phosphatase levels and mild to moderate hyperbilirubinemia. The abnormalities typically develop during the first two postoperative weeks and occasionally may take weeks or months to resolve. The diagnosis of benign postoperative cholestasis should be considered in patients after major or complicated abdominal or thoracic surgery. Benign postoperative cholestasis may also occur in patients who receive large amounts of transfusions, develop a postoperative infectious process, or undergo surgery in the setting of burns or trauma.

The pathogenesis of benign postoperative cholestasis is not fully understood but it is generally considered a multifactorial process. Factors such as excessive bilirubin production from hematomas or transfusions, infections, prolonged complex surgery, hypoxemia, and decreased hepatic perfusion may play a role in the development of benign postoperative cholestasis. At the cellular level, cytokines resulting from the systemic inflammatory response may interfere with canalicular bile transport and lead to cholestasis (21).

Because there is no specific test to confirm benign postoperative cholestasis, this diagnosis is based on the exclusion of other causes of jaundice, most specifically obstruction. Basic viral hepatitis serologies are necessary if there is prominent ALT level elevation (greater than five to ten times of the upper normal limit) associated with cholestasis. Surgical and anesthesia records may reveal prolonged arterial hypotension, intraoperative heart failure, or sustained hypoxemia in patients who develop benign postoperative cholestasis. Liver biopsy is not required to establish the diagnosis but it may be considered when atypical features are present, or in patients suspected of having underlying cholestatic liver disease not identified preoperatively. If a liver biopsy is performed, histologic findings supportive of benign postoperative cholestasis include canalicular bile plugs and prominent bile staining, especially in the centrilobular areas; foamy degeneration of hepatocytes; and modest focal steatosis (42). Less frequently, serum bilirubin level may peak between 10 and 40 mg/dL. In these instances, repeated assessment of the biliary tract with ultrasonography is necessary to exclude intercurrent biliary obstruction. The prognosis of benign postoperative cholestasis is variable and depends less on the cholestatic reaction than on the

overall condition of the patient and severity of associated comorbidities. Management is directed toward the reversal of factors leading to impaired hepatic function and include appropriate intravascular volume support, correction of anemia, antibiotic therapy for infections, and replacement of potentially cholestatic therapeutic drugs by agents metabolized and excreted by extrahepatic sites. There are no data supporting the routine use of choleretics such as ursodeoxycholic acid to treat or expedite the resolution of benign

postoperative cholestasis. When the cholestasis is prolonged, the associated postoperative decreased enteral nutrition and use of antibiotics may cause vitamin K deficiency and lead to coagulopathy. Parenteral administration of vitamin K is useful in correcting the prolonged prothrombin time in this setting. Failure of vitamin K to correct the prothrombin time should raise the suspicion of underlying parenchymal liver disease.

Hepatic Ischemia

Ischemic injury to the liver is a relatively common cause of postoperative abnormal liver function test results (26,27,28). There are multiple mechanisms by which the liver may experience perioperative ischemic damage, including arterial hypotension leading to diminished splanchnic arterial perfusion and portal vein blood flow, effect of anesthetic agents, right or left cardiac failure, severe hypoxemia, and even ischemic-reperfusion injury. Moreover, the patient with cirrhosis is especially susceptible to intraoperative ischemia because of the increased dependency of the cirrhotic liver on hepatic artery blood flow. Whether patients with NAFLD or NASH are more prone to perioperative hepatic ischemia is not known. A recent report on bariatric surgery in patients with NASH-related cirrhosis did not find increased postoperative ischemic complications (4).

Jaundice may occur in severe cases of hepatic ischemia but more often the injury is characterized by an early and rapid rise of the levels of both aminotransferases within 1 to 10 days of the surgical procedure. The extent of the elevation varies considerably and may range from 2 to greater than 100 times the upper normal limit of AST and ALT concentration. Once the hepatic hypoperfusion is corrected, ALT and AST levels also generally begin to decline at a fast rate. Alkaline phosphatase level is generally normal or only mildly elevated. LDH level is also markedly elevated in hepatic ischemia. It is important to consider that in postoperative trauma patients, AST, ALT, and LDH may not necessarily reflect hepatic injury but these enzymes may also originate from extensive muscle injury or rhabdomyolysis. In severe cases of hepatic ischemia, elements of liver failure may develop, such as coagulopathy, hypoglycemia, and hepatic encephalopathy. However, hepatic ischemia is an uncommon etiology of acute liver failure (43).

The diagnosis of postoperative hepatic ischemia is based on the clinical picture, the time course and pattern of liver biochemical abnormalities, and the identification of perioperative conditions leading to hepatic ischemia. Therefore, a history of intra- or postoperative myocardial infarction, shock, pulmonary embolism, significant hypoxemia, or arterial hypotension of any cause will often be found associated with postoperative ischemic hepatic dysfunction. A review of the intraoperative anesthesia records may reveal periods of sustained

arterial hypotension. If the surgical procedure consisted of a cholecystectomy or surgery near the *porta hepatis*, or involved an anatomic hepatic resection, the possibility of inadvertent ligation of the hepatic artery or a main branch must be considered. Imaging studies (e.g., magnetic resonance imaging [MRI], multiphase computed tomography [CT], and Doppler ultrasonography) may show areas consistent with infarction or absent hepatic vascular flow.

Given the predominant elevation of levels of aminotransferases, the differential diagnosis must include viral hepatitides and drug-induced hepatitis. However, the increase in the levels of these liver enzymes generally occurs later in the postoperative period, and the rise and decline in their concentration are more gradual than the relatively rapid changes observed in hepatic ischemia. Liver biopsy is not necessary to establish the diagnosis of ischemic hepatic injury except when the clinical picture is obscure. Histologic findings of noninflammatory centrizonal hepatocellular necrosis support the diagnosis of postoperative hepatic dysfunction due to ischemic injury.

Management of postoperative hepatic dysfunction due to ischemic injury consists of restoration of hepatic perfusion by appropriate support of the intravascular blood volume.

Hypoxemia should be corrected and the underlying complications (i.e., hemorrhage, cardiac failure, pulmonary embolism, and sepsis) treated accordingly. The outcome of hepatic ischemia is generally favorable but the patient's prognosis is determined by the severity of the associated comorbid conditions.

Postoperative Jaundice due to Increased Bilirubin Load

Mild degrees of unconjugated (indirect-reacting) bilirubin are not uncommonly observed in postoperative patients. Although the liver has a large capacity to take up, conjugate, and excrete bilirubin, under certain circumstances, the amount of bilirubin produced may exceed the capacity of hepatic removal. Therefore,

resorption of large hematomas, large volume transfusion, and underlying hemolytic conditions (e.g., glucose-6-phosphate dehydrogenase [G6PD] deficiency, sickle cell disease, autoimmune hemolytic anemia, and thalassemias) may result in excessive production of bilirubin. In other patients, increased intravascular hemolysis is caused by therapeutic drugs, mechanical heart valves, or infections. The diagnosis should be suspected when mild to moderate hyperbilirubinemia (2 to 6 mg/dL) develops during the first two postoperative weeks. Because of the considerable content of AST and LDH in red blood cells, the hyperbilirubinemia may be accompanied by modest elevation in the levels of AST and/or LDH. ALT level, alkaline

phosphatase level, prothrombin time, and serum albumin level should be normal or only minimally altered. Deformed red blood cells in the peripheral smear, low circulating haptoglobin levels, and increased reticulocyte count point to hemolysis as the cause of postoperative jaundice.

Frequently, patients have multiple factors that may cause postoperative jaundice, including hepatic ischemia, underlying liver disease, infection, and impaired renal function. In this multifactorial setting, the effect of bilirubin overproduction is compounded by hepatic dysfunction. Serum bilirubin levels may reach higher values, have a substantial component of conjugated bilirubin, and may be accompanied by significant elevation in the level of ALT and coagulopathy.

Management consists of close monitoring of the liver biochemical abnormalities while correcting the reversible factors outlined earlier.

Postoperative Jaundice Associated with Parenteral Nutrition

Total parenteral nutrition (TPN)-induced liver dysfunction is not uncommon; however, it is rarely the primary cause of postoperative jaundice. Other etiologies of liver dysfunction must be ruled out before this diagnosis. In fact, the indication for TPN is often associated with significant comorbidities that may lead to postoperative hepatic dysfunction. Discerning the length of time for which the patient has received TPN is another significant factor. Chronic TPN administration, usually for more than 3 months, may lead to the development of liver disease. Bacterial or fungal sepsis is associated with both cholestasis and TPN administration. Bacterial overgrowth from bowel disease and the presence of indwelling catheters predispose patients to systemic infection. Conversion to enteral nutrition as early as possible should be the primary goal.

Within the first 2 weeks of TPN administration, mild elevation of liver biochemical test results is frequently observed. Benign steatosis is the most common finding on biopsy and is often the result of excessive caloric intake, particularly from dextrose (44). Rarely, patients may present with right upper quadrant pain and hepatomegaly (45). The initial step in evaluating abnormal liver biochemical test results in a postoperative patient on TPN should include an assessment of the percentage of calories from carbohydrates and fats and adjustment of the formulation as necessary.

The mechanism for TPN-induced steatosis is multifactorial. Hormonal derangements and nutritional deficiencies that promote production of fat and impair its release from the hepatocyte are involved. Although controversial, several reports support the use of oral lecithin

supplements and choline-supplemented TPN to ameliorate steatosis (45,46). Benign steatosis is associated with a moderate increase in aminotransferase levels along with mild increases in alkaline phosphatase and serum bilirubin levels. These laboratory abnormalities are nonspecific and are present in other etiologies of postoperative liver dysfunction. Therefore, liver biopsy may be helpful when the diagnosis is uncertain. Histology can range from isolated periportal fat to panlobular involvement in severe cases (47,48). The laboratory and histologic changes generally correlate with length of TPN administration. Withdrawal of TPN often leads to complete resolution. If a postoperative patient is TPN dependent, however, manipulation of the TPN formula or cycling over 10 to 12 hours each day may allow for reduction or slowing of the process. As TPN administration is prolonged, steatohepatitis with eventual fibrosis may develop (49). Withdrawal of TPN at this point may or may not lead to improvement in fibrosis. However, many of these patients have intestinal failure and are TPN dependent. These patients may ultimately require combined liver–intestinal transplantation. TPN-induced cholestasis is a less common finding in short-term courses of nutritional support. If it does occur, it may present as early as 3 weeks after starting therapy. In children, cholestasis is the most common presentation and is related to incomplete development of biliary secretory mechanisms in premature infants (50). In adults, cholestasis manifests itself as a slow and progressive elevation of alkaline phosphatase and bilirubin levels. In these patients, early use of metronidazole to prevent bacterial overgrowth and ursodeoxycholic acid to induce bile flow and promote a more hydrophilic bile may help improve the hepatic dysfunction (51,52). Lastly, TPN may directly affect the biliary tract. Patients who are unable to use their gastrointestinal system develop akinesis of the gallbladder with biliary stasis and ultimately develop sludge. Length of TPN therapy correlates with gallbladder dysfunction. After 6 weeks, most patients have evidence of gallbladder sludge on ultrasound (45,53). The presence of sludge promotes the production of biliary stones,

thereby leading to the complications associated with cholelithiasis. Regardless of sludge or stones, TPN may play a role in acalculous cholecystitis. This diagnosis is usually made in critically ill patients, with multiple factors contributing to its development. Because the diagnosis is difficult to make and the morbidity is significant, prevention is the primary goal. Early enteral feeding or administration of cholecystokinin to stimulate gallbladder activity appears beneficial. A calculous cholecystitis can also occur in chronically ill patients who have been TPN dependent for longer than 3 months. The patients may need a cholecystectomy if medically stable or otherwise drainage by a percutaneously placed cholecystostomy tube (54).

Postoperative Jaundice following Liver Transplantation

With current liver allocation based on the MELD score, many patients undergoing liver transplantation have prolonged jaundice in the posttransplantation period. The differential diagnosis for postoperative onset or worsening of jaundice after liver transplantation is broad and include primary allograft dysfunction, ischemic injury, intraoperative transfusions, therapeutic drug hepatotoxicity, biliary and/or vascular complications, hematoma reabsorption, acute cellular rejection, ABO mismatch, disease recurrence, and bacterial, fungal, and viral infections. The evaluation and management of postoperative liver allograft dysfunction is reviewed in detail elsewhere in this book (see Chapters 55 and 56).

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Chapter 26

The Hepatitis Viruses

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Key Concepts

- *Hepatitis A virus* is a ribonucleic acid (RNA) virus accounting for most cases of acute hepatitis. Seroprevalence rates in industrialized countries have been falling because of vaccination programs and improvements in socioeconomic conditions. As a result, an age-related shift in seroprevalence has taken place in which adults and elderly are presently more at risk of infection.
- *Hepatitis B virus* (HBV) is a deoxyribonucleic acid (DNA) virus, with the highest prevalence rates of infection in Southeast Asia, China, and Africa. Most persons who acquire the infection before 5 years of age develop chronic hepatitis B infection. The natural course of chronic infection is variable. HBV-infected patients have an increased risk of developing hepatocellular carcinoma (HCC) compared to uninfected patients. A variety of therapies are available and approved, including conventional interferon (IFN), pegylated IFN, and nucleoside analogs such as lamivudine, adefovir dipivoxil, and entecavir. Treatment of HBV is, however, still limited by factors such as the development of drug resistance and the low efficacy of available therapies in eliminating covalently closed circular HBV DNA.
- *Hepatitis C virus* (HCV) is an RNA virus, accounting for most patients with chronic hepatitis worldwide. Intravenous drug use is presently the primarily source of infection. The natural course of HCV infection, although slowly progressive usually, is variable. Pegylated IFN has been shown to be effective in permanently eradicating the virus in the setting of acute HCV infection. Presently, the combination of pegylated IFN-α and ribavirin is the standard therapy for chronic HCV infection and appears to cure approximately 50% of treated patients. Response and duration of treatment is dependent on the infecting viral genotype.
- *Hepatitis delta virus* (HDV) relies on HBV for infectivity and persistence. Dual infection with HDV and HBV results in progressive liver disease and increased risk of HCC. IFN remains the only drug to

have any therapeutic effect.

- *Hepatitis E virus* (HEV) is an RNA virus that causes an acute self-limiting hepatitis with no chronic sequelae. Acute HEV infection, however, results in a high mortality rate among pregnant women, particularly during the third trimester.
- *Hepatitis G virus* (HGV) is a recently identified RNA virus. A firm and direct association with liver pathology is still lacking, and it is unclear whether HGV is hepatotropic. Coinfection with HGV and human immunodeficiency virus (HIV) has been shown to improve mortality and morbidity for the HIV-infected individuals and slows progression to acquired immunodeficiency syndrome.
- Herpesviruses are a family of large enveloped DNA viruses. Infection with herpesviruses occurs worldwide. Herpesvirus infection is occasionally associated with hepatitis. Clinically overt severe and fulminant hepatitis may, however, occur, usually in immunocompromised hosts.

There are six identified human hepatitis viruses; these are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis delta virus (HDV), hepatitis E virus (HEV), and hepatitis G virus (HGV). These viruses have distinctive biologic and clinical properties that have been unraveled over the years. In this regard, substantial advances have been made in our understanding of the genomic structure, life cycle, natural history, and pathogenesis of most of these viruses. We have also been able to exploit our increased knowledge to develop effective therapeutic strategies for each of these viruses, and several advances have been made in this regard (Table 26.1). Despite these ongoing efforts, viral hepatitis continues to represent a major public health challenge worldwide. Also, chronic hepatitis remains a disease with significant morbidity and mortality, and response to current therapy is far from optimal.

This chapter introduces each of these viruses, giving an overview of the epidemiologic aspect, molecular aspect, natural history, immunopathogenesis, and treatment aspect of each. In addition, we discuss briefly the herpesviruses, which are not primarily hepatotropic, but are occasionally associated with hepatitis.

Hepatitis A Virus

Epidemiology

HAV infection is a major cause of acute hepatitis and liver failure throughout the world. HAV infection is particularly common in developing countries of Africa, Asia, and Latin America, where seroprevalence rates approach 100% (1). In these countries, most infections occur by 5 years of age. By contrast, seroprevalence rates in industrialized countries have been falling because of improvements in socioeconomic conditions. As a result, an age-related shift in seroprevalence has taken place, so that adults and

the elderly are at a greater risk of acquiring an HAV infection. Therefore, about one third of the US population has serologic evidence of previous HAV infection, with prevalence rates ranging from 9% among children to 75% among persons aged 70 years or older (1).

HAV is primarily transmitted enterically (fecal–oral route), typically by ingestion of contaminated food or water and through person-to-person contact within the household (2). Crowded or unsanitary conditions are commonly implicated. Although it is rare, parenteral transmission due to the use of contaminated blood products is possible, while vertical transmission is extremely uncommon (2,3). Risk groups for HAV infection include travelers to endemic areas, military personnel, day care workers, and the institutionalized patients. Also, homosexuals with multiple sexual partners and intravenous drug users are at higher risk for HAV acquisition (2).

Table 26.1. Recent Developments in Therapy for Viral Hepatitis

HAV
Effective vaccines are available; however, HAV remains a major cause of acute hepatitis worldwide
HBV
Effective vaccines have reduced HBV prevalence in many countries
Pegylated interferon monotherapy has recently been shown to have significant virologic efficacy in a trial comparing this treatment alone to combination of pegylated interferon and lamivudine or lamivudine alone in largely Asian HBeAg-positive patients
In patients awaiting liver transplantation, lamivudine has been shown to suppress HBV DNA, stabilize the progression of liver disease, delay the need for liver transplantation, and improve survival among these patients
Adefovir dipivoxil suppresses HBV DNA in patients with lamivudine resistance
In HBeAg-negative patients, the virologic response achieved by a 12-mo course of adefovir is not sustained once treatment is ceased
Entecavir effectively suppresses HBV DNA in HBeAg-positive and HBeAg-negative patients, as well as in those with lamivudine resistance
HCV
Pegylated interferon monotherapy is effective in achieving viral clearance in acutely infected patients
Combination pegylated interferon and ribavirin is presently the treatment of choice for chronic HCV infection
Combination pegylated interferon/ribavirin is well tolerated in HCV/HIV coinfected patient and results in acceptable virologic response rates

Combination pegylated interferon/ribavirin is presently used to individually treat significant recurrent HCV infection after liver transplantation
Ongoing research into novel therapeutic agents and vaccine development is proceeding rapidly

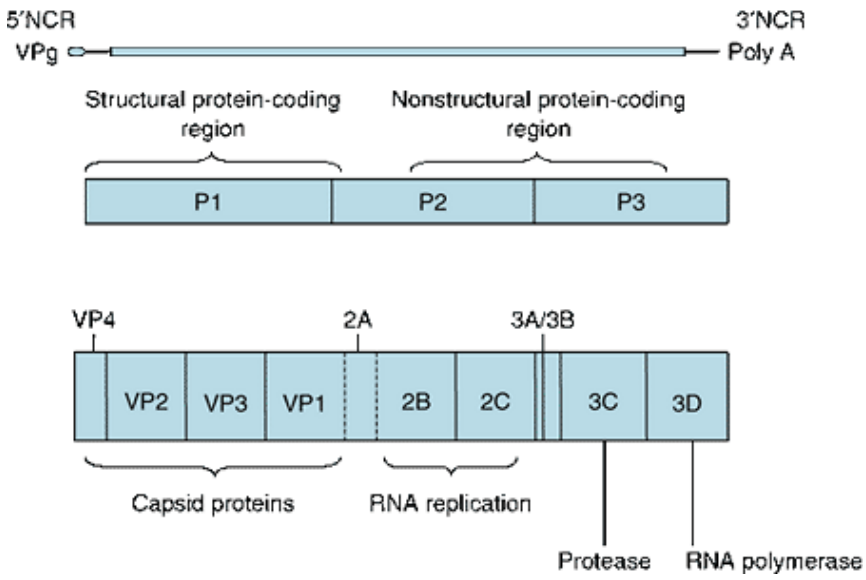
HDV

High-dose prolonged interferon monotherapy remains the most effective therapeutic option, although side effects may limit therapy

HAV, hepatitis A virus; HBV, hepatitis B virus; HBeAg, hepatitis B e-antigen; DNA, deoxyribonucleic acid; HCV, hepatitis C virus; HDV, hepatitis delta virus; HIV, human immunodeficiency virus.

The Hepatitis a Virus Genome and Proteins

HAV is a ribonucleic acid (RNA) virus, a member of the Picornaviridae family. HAV is a nonenveloped icosahedral particle 27 to 32 nm in diameter (4). The infectious particle consists of a capsid protein and the RNA genome. The HAV genome is a positive single-stranded RNA approximately 7.5 kb long (Fig. 26.1). The genome is defined by three distinct regions: The 5′ noncoding region (NCR) of approximately 735 nucleotides contains an internal ribosomal entry site (IRES). As with other picornaviruses, the 5′ end of the genome does not have a cap structure but instead is covalently linked to the viral protein VPg, involved in the initiation of RNA synthesis. The 5′ NCR appears to be the most conserved region of the genome. Some mutations in the 5′ NCR region enhance growth of HAV in cell culture but do not appear to have a major role in determining HAV virulence. The 5′ NCR is followed by a single open reading frame (ORF) for a large polyprotein containing P1, P2, and P3 regions. The P1 region encodes the capsid proteins VP1 to VP4. Mature capsid proteins are the predominant form in virions. VP1 and VP3 form a single, dominant epitope on the viral capsid and elicit a neutralizing antibody response. VP4 harbors a potential myristoylation site and is essential for virion formation. The P2 and P3 regions encode the nonstructural proteins associated with viral replication. Among these proteins, 3C is recognized as the sole protease for HAV, while protein 3D has RNA-dependent RNA polymerase activities. Precursor polypeptides include VP0, which is a VP4/VP2 fusion protein. Finally, the 3′ NCR is about 63 nucleotides in length, terminating in a poly-A tract.



▪ **Figure 26.1** The hepatitis A virus (HAV) genome—the HAV genome is a linear, positive-sense, single-stranded ribonucleic acid (RNA) approximately 7.5 kb in length. A VPg protein is covalently attached to the 5′ end of the genome and a poly-A tail at the 3′ end. The genome codes for a single polyprotein of approximately 2,235 amino acids. The polyprotein is divided into three main functional domains: P1, P2, and P3. P1 encodes the viral capsid proteins while P2 and P3 code for the nonstructural proteins including RNA helicase, protease, and RNA polymerase. 5′ NCR, 5′ noncoding region.

Hepatitis a Virus Life Cycle

It is not clear how the HAV reaches the hepatocytes. The virus is taken up by a mucin-like class I integral membrane glycoprotein, presumed to be the receptor for HAV (5). However, it is not known how the virus reaches the cytoplasm, the site of viral replication. Replication of the genome occurs by a mechanism involving the RNA-dependent RNA polymerase. In this regard, virus-encoded proteins replicate the RNA genome through a negative-strand intermediate. A relatively large number of plus strands are transcribed from the minus strand. The new plus strand may enter another cycle of replication or serve as a template for polyprotein translation. For translation, cis-acting picornaviral IRES is thought to interact with trans-acting cellular factors, so that 40S ribosomal subunits begin translation at the correct initiation codon. The HAV has two initiation codons that can function independently (6). The single ORF of the picornaviruses is translated into one long precursor polypeptide (Fig. 26.1), which is processed by a cascade of proteolytic cleavage to yield the mature viral proteins (7). 3C protein is the primary cleavage enzyme. The primary cleavage takes place cotranslationally at the C-terminus of the VP1/2A precursor. Subsequently, 3C secondarily catalyzes VP2/VP3 and VP3/VP1 to

produce 14S pentamers containing VP0, VP3, and VP1/2A. VP1 is released from 2A by cellular proteases. Maturation

cleavage involves the processing of VP0 to release VP4 and VP2. Trans-acting host factors for HAV may play a role in the translation process. The pentamers assemble to form empty capsids (procapsids) or incorporate virion RNA to form provirions. The mechanisms by which the virus is released and secreted from the infected cell have not been entirely elucidated as yet.

Natural History of Hepatitis a Virus Infection

Humans are the principal host of HAV. Most infections (70%) in children younger than 6 years are asymptomatic, in contrast to a frequently symptomatic course in adults. With increasing age, infections become more and more clinically apparent, and by adolescence, more than 80% of patients develop jaundice (2,8). After acute HAV infection, recovery with lifelong immunity is the norm. Uncommon clinical features of acute HAV infection include the development of cholestatic hepatitis or, less commonly, relapsing hepatitis. In each of these situations, however, the prognosis is good. Fulminant hepatitis develops in less than 1% of acutely infected HAV cases and is more common among infected adults. Therefore, elderly patients and persons with chronic liver disease are at increased risk of progressing to fulminant liver failure (9). Although spontaneous recovery usually occurs, liver transplantation may be necessary for those patients who fail to improve.

Immunopathogenesis of Hepatitis a Virus Infection

HAV is unlikely to be a cytopathic virus, and the host immune response to HAV is believed to be responsible for the observed necroinflammatory lesion. In support of this, increased expression of CD4 and $CD8^+$ T-cell receptors has been demonstrated at inflammatory sites in the livers of acutely infected patients (10). Moreover, HAV-specific $CD8^+$ T cells with cytotoxic activities have been isolated from these affected livers (11). In the acute phase of the illness, a dominant immunoglobulin M (IgM) and IgG immune response against VP1 has been identified. Similarly, antibodies against VP3 and VP0 develop during the late convalescence phase. Circulating antibodies, therefore, seem to limit the viremic phase. Nonsecretory IgG antibody to HAV then persists in the serum after infection, providing lifelong immunity.

Treatment of Hepatitis a Virus Infection

No specific therapy is required for acute HAV, and antiviral therapy is not available. The most effective means of controlling HAV are preventive measures relying on strict hygiene and vaccination programs. Available vaccines are safe, highly immunogenetic, and induce long-lasting (>20 years) protection against HAV. Routine vaccination is currently recommended for high-risk groups aged 2 years or older. These groups

include young children in endemic areas and travelers to these areas, homosexual men, intravenous drug users, and patients with chronic liver disease (12).

Hepatitis B Virus

Epidemiology

The epidemiology of HBV infection varies greatly worldwide. The HBV carrier rate ranges from 8% to 20% in highly endemic areas such as Southeast Asia, China, and Africa to less than 2% in North America, western Europe, and Australia (13). In highly endemic areas, spread of HBV infection is mostly the result of maternal–infant transmission (vertical), while the use of reusable syringes and injectable drugs, and sexual spread, are also important. In contrast, sexual activity and injectable drug use account for most HBV cases in low endemic areas. However, even in low endemic areas, HBV infection is present in a substantial number of immigrants from highly endemic areas. Other less common risk factors for HBV transmission include occupational exposure, hemodialysis, acupuncture, household contact, and the receipt of infected organs or blood products (13).

In several countries, effective strategies have been implemented to reduce the risk of HBV transmission. These include public education, routine infant and adolescent vaccination, HBV screening of pregnant women, and administration of postexposure prophylaxis to infants of infected women (14). In Taiwan, the HBV vaccination program in newborns has dramatically decreased both the prevalence of hepatitis B infection and the incidence of hepatocellular carcinoma (HCC) in this population (15).

Hepatitis B Virus Genome and Proteins

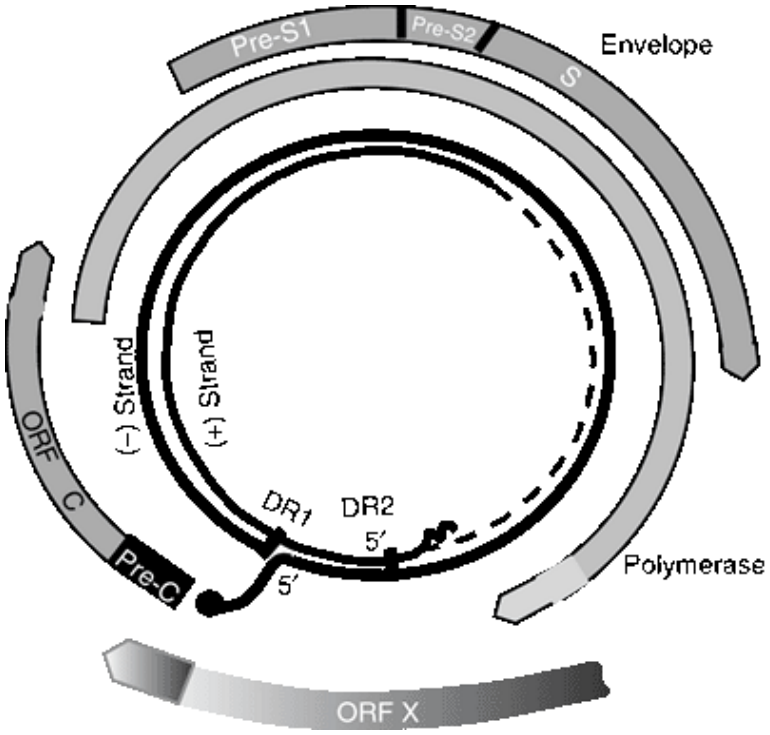
HBV is a member of the Hepadnaviridae family (hepatotrophic deoxyribonucleic acid [DNA] viruses). The mature HBV virion, also known as the *Dane particle*, is 42 nm in diameter, consisting of an outer lipoprotein layer that encodes the viral envelope proteins, the hepatitis B surface antigen (HBsAg), and surrounds a nucleocapsid core, the hepatitis B core antigen (HBcAg) (16). The nucleocapsid contains the viral genome and the viral polymerase. In addition to the mature virions, HBV-infected serum contains two other distinct subviral particles that are either spherical

or filamentous in shape and are approximately 20 nm in width. Subviral particles reach a 10,000-fold higher concentration than virions in the serum. They consist of an envelope glycoprotein and host-derived lipids. The precise biologic significance of this massive overproduction of empty envelopes is unknown; however, it has been speculated that they serve as decoys for the host's immune system.

The HBV genome (Fig. 26.2) is a partially relaxed double-stranded circular DNA of approximately 3,200 base pairs (bp) and encodes four partly overlapping ORFs: The envelope or the surface gene, the core gene, the

polymerase, and the X gene (HBx). The 5′ end of the complete minus DNA strand is covalently linked to the polymerase-coding gene, while the 5′ end of the plus strand is linked with a short piece of capped RNA. The termini of the 5′ end of the minus and plus strands map to the regions of short (11 to 12 nucleotides) direct repeats (DRs) in viral DNA, known as *DR1* and *DR2*. These repeat regions are involved in priming the synthesis of their respective DNA strands.

The viral envelope consists of cellular phospholipids and three virally encoded proteins, the small (S) or major, the middle (M), and the large (L) polypeptides. The L, M, and S proteins share a highly hydrophobic membrane region referred to as the *S domain*. Additionally, the L protein has a long region of 109 to 120 amino acids called *pre-S1*. The M protein has a 55 amino acid region identified as the pre-S2. Translation of these envelope proteins is initiated at three different in-frame start sites within a single ORF and is ended at a common termination codon (17).



▪ **Figure 26.2** The hepatitis B virus (HBV) genome—the viral deoxyribonucleic acid (DNA) is partially double stranded. It encodes seven proteins from four open reading frames (ORFs)—envelope (surface), core, polymerase, and the X gene and three upstream regions: Precore, pre-S1, and pre-S2. A polymerase protein is covalently attached to the minus (–) strand and a capped oligoribonucleotide is attached to the plus (+) strand. Direct repeats 1 and 2 (DR1 and DR2) are 11 base pairs repeat sequences with template functions during replication.

The core gene consists of the precore and core regions. The precore–core region encodes HBcAg and hepatitis B e-antigen (HBeAg) (18). The production of HBcAg is derived by an initiation AUG codon in the core gene, whereas HBeAg requires translational initiation from an AUG codon upstream of the core gene on the precore messenger RNA (mRNA). This 25-kDa precore–core fusion protein is processed by two cleavage events to generate a final circulating 16-kDa HBeAg fragment. Mutations emerging in the precore and core promoter regions result in reduced levels of HBeAg expression (19). Similarly, a recent novel class of mutations immediately preceding the precore AUG codon has been shown to reduce HBeAg expression by a leaky scanning mechanism facilitating early HBeAg seroconversion in carriers of the virus (20). Expression of HBeAg has been shown to be nonessential for virus viability in animal models and humans (17), and its precise biologic role in the life cycle of the virus remains unclear. HBeAg may serve an immunoregulatory function in the infected host.

The polymerase-coding region is specific for the viral polymerase involved in DNA synthesis, as will be discussed in the following sections.

The viral X gene codes for a 16-kDa protein termed the *HBx*, which exhibits pleiotropic activities. Absence of functional HBx in vivo has been shown to reduce core gene expression, supporting the hypothesis that an essential function of HBx is to enhance obligatory virus gene expression during infection (21). HBx also plays an important role in modulating host cell signal transduction and activating several cellular signaling pathways. Distinct from the transactivation, HBx modulates the DNA repair processes and as such has been implicated in the pathogenesis of HCC (22).

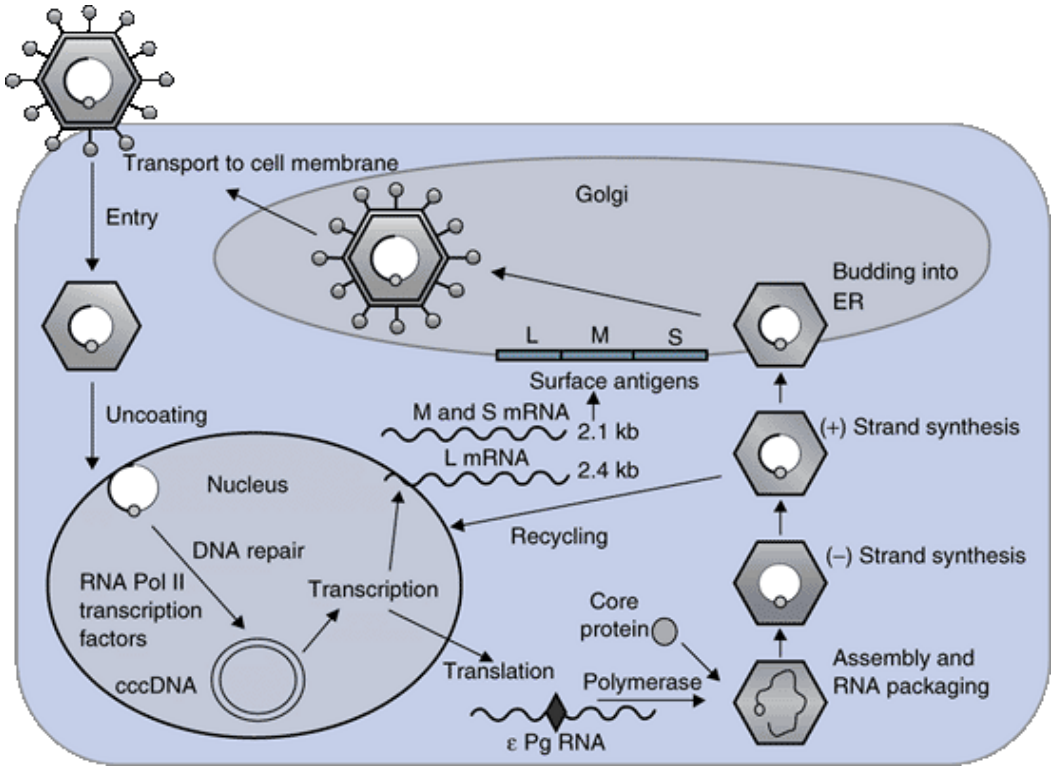
Hepatitis B Virus Life Cycle

The life cycle of HBV (Fig. 26.3) has been elucidated from studies in animal models and tissue cultures.

Entry and uncoating

The cycle begins by viral attachment to the host cell membrane through the envelope protein. Although the precise identity of the actual cellular receptor(s) remains unknown, the pre-S1 domain in the L-envelope region appears to be directly involved in the viral attachment to the host cell surface (23). The heat shock protein (hsp) 70 may also be involved in this process (24). After membrane fusion, the envelope is stripped

off and the nucleocapsid is released into the cytoplasm. Little is known about the postbinding steps in viral entry. It is likely, however, that the capsid "escorts" the viral DNA to the nucleus. Once in the nucleus, the partially double-stranded DNA is converted (or repaired) by viral polymerase into covalently closed circular DNA (cccDNA). This requires removal of its terminal structures (RNA and P protein), repair of the single-stranded gap region, and covalent ligation of the DNA termini.



▪ **Figure 26.3** Schematic representation of the life cycle of hepatitis B virus (HBV) (see text for explanation). L, M, and S are large-, middle-, and small-surface envelope proteins, respectively; ER, endoplasmic reticulum; RNA, ribonucleic acid; cccDNA, covalently closed circular deoxyribonucleic acid; mRNA, messenger ribonucleic acid; Pol II, polymerase II; Pg, pregenomic.

Transcription

Nuclear cccDNA serves as the template for transcription by host RNA polymerase II. Persistence of the virus in infected cells during chronic infections is likely to be dependent on generating, maintaining, and regulating the pool size of transcriptionally active cccDNA molecule.

Two classes of transcripts are synthesized from cccDNA, the genomic and the subgenomic classes. Both classes contain several transcripts, all unspliced and polyadenylated at a common position within the core gene. The subgenomic transcripts function as mRNA for the translation of the envelope proteins (L, M, and S proteins) and HBx protein (25). The two genomic mRNAs encode the precore, core, and polymerase ORFs.

The HBV DNA contains promoters corresponding to independent transcription from pre-S1, pre-S2/S, core, X, and possibly, precore. The HBV pre-S1 promoter directs transcription of the 2.4-kb large envelope, while the S promoter directs the transcription of several mRNAs of about 2.1 kb, which are translated to produce pre-S2 (middle) and S (small)

HBsAg proteins. The core promoter directs the transcription of pregenomic mRNA and precore mRNA. The precore mRNA translates the precore protein, while the pregenomic RNA serves as the template for reverse transcription. The pre-S1 and precore promoters are exclusively liver specific.

Two regions of the HBV DNA are identified to have the properties of transcription enhancers (26,27). These enhancers stimulate transcription in cis configuration in a position- and orientation-independent way. Enhancer I (En I) is located between the S and X ORF, while En II is located just upstream of the core promoter. The activity of En II is largely restricted to hepatic cells and is therefore responsible for activating viral replication in a liver-specific manner (28). In contrast, En I functions in hepatic and nonhepatic cells, strongly upregulating all the major HBV promoters (29,30).

Genomic replication

The first step in replication involves incorporation of viral RNA into the core, together with the polymerase. This encapsidation process is highly selective. The main trigger of this process is binding of the polymerase protein to a 5′-proximal stem loop structure called ε

(which acts as an encapsidation signal) on the pregenomic RNA. Mutations in the polymerase ORF allows capsid formation but not RNA encapsidation. This reaction also requires host proteins such as chaperone hsp90 (31). Encapsidation then proceeds by packaging the viral pregenomic RNA bound to the viral polymerase protein into core particle (32) together with hsp90, forming the nucleocapsid (33).

Viral deoxyribonucleic acid synthesis

Within the viral nucleocapsid, reverse transcription of the pregenomic RNA occurs. The pregenomic RNA is terminally redundant (200 nucleotides), containing the ε stem loop and a copy of the short DR1 sequence. There is growing evidence that initiation of the viral DNA minus strand synthesis occurs within ε and not DR1 as initially thought (34,35). This is initiated by the binding of polymerase to ε, generating a polymerase-linked oligonucleotide of about four nucleotides. These four nucleotides are homologous to the four nucleotides within DR1, allowing DNA transfer and annealing to this position. The strand transfer reaction occurs efficiently and is selectively directed to the 3′ copy of DR1. Once this occurs, elongation of the minus strands proceeds to the end of the template, completing minus strand synthesis. The minus DNA strand serves as the template for synthesis of the plus DNA strand. Plus-strand synthesis is initiated at DR2 and primed by capped oligonucleotides derived from the 5′ of pregenomic RNA. Once cleaved, the new RNA primer is translocated to the 5′ end of the minus strand to base pair with the DR2 region, forming the plus strand. The steps involved in the translocation process are poorly understood. Elongation of the plus strand then proceeds to the 5′ end of the minus strand DNA template, at which point the template becomes

exhausted. At this stage, another transfer is required to complete the plus-strand synthesis. This transfer is made to the 3’ end of the minus strand DNA and is facilitated by eight nucleotides of the sequence derived from the terminal redundancy in the pregenomic RNA. Annealing of the nascent plus-strand DNA to the 3’ end of the minus strand circularizes the DNA and allows further elongation. Finally, the relaxed circular double-stranded DNA virion (the mature DNA) is formed, with the 5’ end covalently bound to a primer protein. The resulting nucleocapsid either moves back to the nucleus and recycles its genome, thereby replenishing the cccDNA pool (36), or buds into the postendoplasmic reticulum where virus envelopment occurs.

Viral assembly and release

S proteins are synthesized in the rough endoplasmic reticulum and then transported to the postendoplasmic reticulum and pre-Golgi regions, where budding of the nucleocapsid occurs. A linear sequence in pre-S domain (residues 103 to 124) of the L envelope protein has been implicated in HBV envelopment and secretion. Other regions in the S domain are also important in viral secretion. Finally, the assembled HBV virion is transferred to the Golgi to be secreted.

Natural History of Hepatitis B Virus Infection

Primary HBV infection is clinically symptomatic in 30% to 50% of persons older than 5 years, yet most patients (95%) will clear the virus, resulting in lifelong immunity. In contrast, 30% to 50% of persons who acquire the infection before the age of 5 years develop chronic hepatitis B infection.

The natural course of the HBV infection has three phases: The first is the immune tolerance phase seen in children and adolescents who acquire HBV perinatally. In this phase, patients are HBeAg positive and have high serum levels of HBV DNA but normal serum aminotransaminase (alanine aminotransferase [ALT]) levels and minimal histologic activity. The second is the immune clearance phase, occurring in adolescents and adults, during which HBV replication declines, accompanied by both increased serum ALT levels and inflammatory activity in the liver. Seroconversion of HBeAg to anti-HBe may then ensue. During this phase, or even after the seroconversion to anti-HBe, replication-competent HBV variants with mutations in the precore or core promoter regions may emerge, preventing HBeAg production. Of interest, HBeAg-negative disease is now becoming the predominant form of chronic HBV infection (37). In the third low-replicative phase, serum HBsAg persists, but HBeAg is no longer detectable and HBV DNA can only be detected by sensitive polymerase chain reaction (PCR) assays. In this inactive HBsAg carrier state, patients are usually asymptomatic and liver disease is inactive. This stage may last indefinitely; however, a proportion of patients may undergo reactivation with the reappearance of high levels of HBV DNA and/or HBeAg. A recent 30-year follow-up study reported the natural course of HBsAg carriers to be generally favorable (38). In particular, these patients did not seem to develop clinically significant liver disease, hepatocellular cancer, or other liver-related morbidity or mortality at a higher rate than uninfected

controls.

In chronically infected patients, cirrhosis and HCC are the two major complications associated with increased morbidity and mortality. In HBeAg-positive patients, the 5-year cumulative risk of developing cirrhosis ranges from 8% to 20% (39). This incidence is even higher in HBeAg-negative patients. Predictors for the development of cirrhosis include older age,

evidence of persistent viral replication, HBV genotype C, excess alcohol intake, and coinfection with HCV, delta hepatitis, or human immunodeficiency virus (HIV) (40,41). Once cirrhosis is established, the 5-year mortality rate is 16% for patients with compensated cirrhosis but increases to 60% to 80% among those with decompensated cirrhosis. Overall, it is estimated that over 250,000 patients worldwide die annually from HBV-related liver disease (13).

HBV-infected patients have a 100-fold increased risk of developing HCC compared to uninfected patients (42). The risk of HCC correlates with age, gender, HBV-replicative status (HBeAg status), and the severity of the underlying liver disease, particularly the presence of cirrhosis (42,43).

Immunopathogenesis of Hepatitis B Virus Infection

The nature of the immune response is crucial for protection from the disease, viral resolution, and the extent of liver injury in response to HBV infection. During acute infection, innate, humoral, and adaptive immune responses are activated and must act in concert to clear the virus. The earliest events after infection involve noncytolytic control of viral replication through interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). These cytokines are initially produced by infected hepatocytes and then by cells of the innate immune system including natural killer (NK) cells and NK-T cells (44). Formation of neutralizing antibodies to HBV envelope antigens is an important T cell–dependent process that aids viral clearance by interfering with viral entry into host cells. However, for complete viral eradication, a strong, polyclonal, and multispecific CD4 and cytotoxic lymphocyte (CTL) immune response is essential. Self-limited infection is therefore characterized by a vigorous CD4 response directed against multiple epitopes within the HBV antigen, in particular, the nucleocapsid (45). This response also supports the induction and activation of CTLs that mediate apoptosis of infected hepatocytes through Fas/Fas-ligand binding and the release of lytic granules (46). Virus-specific CTLs also trigger the production of other cytokines including TNF-α, IFN-γ, interleukin 2, and NK cells, which control viral replication through noncytolytic mechanisms (47). In those patients who become chronically infected, the immune response, although ineffective, appears to persist and therefore mediates ongoing tissue injury. In this context, CTLs induce both direct damage to hepatocytes and produce cytokines that lead to the recruitment of antigen-nonspecific inflammatory cells (e.g., macrophages, NK cells) and IFN-γ. This nonspecific inflammatory response accounts for most of the

subsequent tissue damage in chronically infected patients (48).

Treatment of Hepatitis B Virus Infection

In the last few years, new therapies have emerged for managing chronic HBV infection. However, a number of unresolved issues remain, including whom to treat, how long should the treatment continue, and what are the optimal treatment regimens to be used in various subsets of patients.

Initial therapeutic regimens in HBeAg-positive patients with chronic hepatitis B consisted of 4- to 6-month courses of IFN-α, at varying dosages between 5 million units (MU) daily and 10 MU thrice weekly, achieving HBeAg loss in 33% of patients, compared with 12% of controls (49). In contrast, in HBeAg-negative HBV patients, a 6-month course of IFN therapy was associated with very low sustained response rates because of frequent relapses after the cessation of therapy. Subsequently, prolonged courses of IFN were reported to improve sustained response rates up to 20% to 25% in these patients (50). The wide spectrum of IFN-related side effects, cost, and the inconvenience of administration are the main drawbacks of this therapy.

Pegylated IFN has better pharmacokinetics than standard IFN, allowing once-weekly dosing. A recent worldwide trial evaluating its efficacy in largely Asian HBeAg-positive patients reported an HBeAg seroconversion rate of 32% after 48 weeks of pegylated IFN monotherapy (51). This seroconversion rate was higher than that achieved in patients receiving either a combination of pegylated IFN and lamivudine (27%) or lamivudine alone (19%) (51).

Lamivudine, a nucleoside analog, resulted in HBeAg seroconversion rates of 17% in patients after 12 months of treatment, at 100 mg daily, compared to 6% in untreated patients (52). It is administered orally and has few side effects. Moreover, longer treatment durations in HBeAg-positive patients enhances response rates, with seroconversion occurring in 27%, 40%, 47%, and 50% of patients receiving treatment for 2, 3, 4, and 5 years, respectively. In HBeAg-negative patients, however, a 12-month course of lamivudine is less effective, maintaining remission (as assessed by undetectable HBV DNA levels) in less than 15% of patients after stopping therapy (53). Importantly, lamivudine has also been reported to suppress HBV replication in 60% to 100% of patients awaiting liver transplantation (54,55). In these patients, lamivudine has been shown to stabilize the progression of liver disease, delay the need for liver transplantation, and improve survival among patients awaiting liver transplantation (55,56,57).

A significant problem with lamivudine is that to achieve the desired therapeutic benefit, longer treatment durations are often required. However, with longer courses of therapy, viral-resistance mutations, particularly the YMDD variant, emerge, with the risk

reaching 50% after 3 years of therapy. The emergence of resistance has been associated with significant and serious flares in disease activity. Unfortunately, very high rates of relapse occur on discontinuing lamivudine

therapy, making it more likely to become a longer-term therapy.

Adefovir dipivoxil is a nucleotide analog of adenosine. In HBeAg-positive patients, a 12-month course of adefovir, at a daily dose of 10 mg, achieved loss of HBeAg in 24% of patients compared to 11% in the placebo group (58). In addition, adefovir has also been shown to be effective in suppressing HBV DNA in patients who develop lamivudine resistance.

In HBeAg-negative patients, initial reports indicated that a 12-month course of adefovir resulted in a significant virologic response in 50% of patients compared to placebo (59). However, a recent follow-up data suggests that this benefit was not sustained once treatment was stopped (60). Side effects are uncommon with adefovir; however, the drug is expensive and renal dysfunction may occur. Resistance to adefovir is being increasingly reported, although it is less common than that with lamivudine and tends to emerge later in the course of treatment (61).

Entecavir is a cyclopentyl guanosine analog. Results of phase III studies confirmed the efficacy and safety of entecavir when given for 48 weeks as compared with lamivudine in suppressing HBV DNA in HBeAg-positive and HBeAg-negative patients and in those with lamivudine resistance (62). A dose–response relationship has been observed. Entecavir is well tolerated at all doses, and resistance to the drug is rare.

Emtricitabine and telbivudine are under evaluation for the treatment of chronic hepatitis B infection. Their clinical utility and approval are awaited.

Hepatitis C Virus

Epidemiology

An estimated 3% of the world's population is infected with HCV (63,64). Globally, HCV infection accounts for 70% of chronic hepatitis cases. The prevalence rate of HCV infection ranges from 20% in highly endemic areas such as Egypt to 1.8% in the United States and 1.5% in Australia.

Parenteral exposure accounts for most HCV infections. With the advent of routine blood screening measures, the importance of HCV transmission from infected blood products has declined. Therefore, injection drug use is the primary source of HCV acquisition, and up to 90% of intravenous drug users are HCV infected (65). Other risk factors include occupational exposure, hemodialysis, medical reuse of infected needles, and tattoos; vertical (mother-to-infant) and sexual transmission are uncommon.

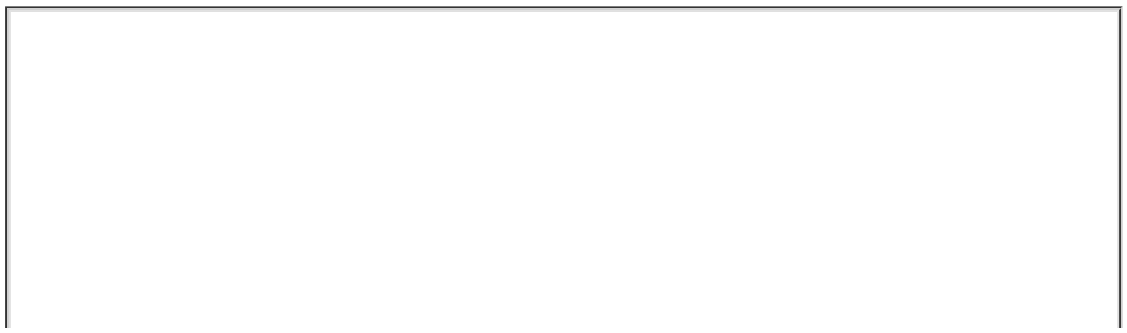
Hepatitis C Virus Genome and Proteins

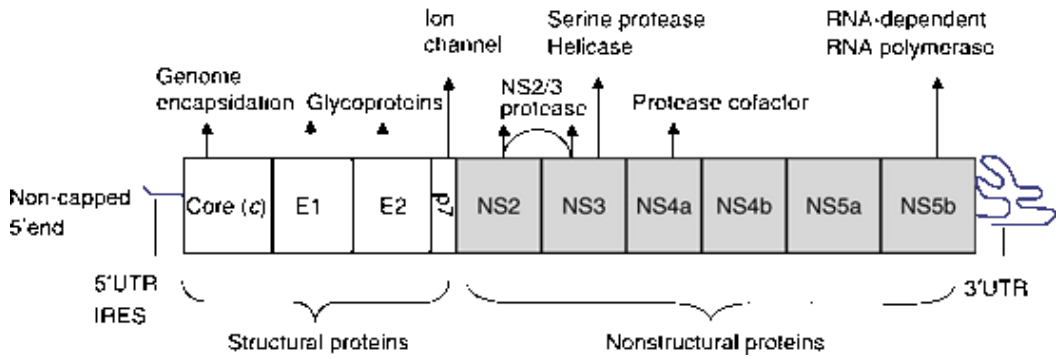
HCV is a member of the Flaviviridae family, of which yellow fever virus and pestiviruses are other members (66). All members of this family are small-enveloped viruses containing a positive-sense, single-stranded RNA genome. The size of the HCV particle is between 30 and 80 nm. The HCV genome is 9.6 kb in length, containing a single ORF flanked by 5′ and 3′ untranslated regions (UTRs) (66) (Fig. 26.4). The 5′ UTR region of the HCV genome contains an IRES for the initiation of translation. This region of

highly conserved nucleotide sequences has been used as the major target for commercial RT-PCR assays. The 3′ UTR region of the genome terminates in polyuridine (poly U) ribonucleotides, followed by another highly conserved region of 98 to 100 nucleotides designated the X tail, which may have functional importance in viral replication (67). The HCV ORF encodes a polyprotein that may vary in length between 3,010 and 3,033 amino acids in a strain-specific manner. Translation of the HCV polyprotein in the endoplasmic reticulum of the infected cell gives rise to at least 10 mature proteins (Figure 26.4). The structural proteins include nucleocapsid protein and two envelope glycoproteins, E1 and E2. This is followed by a small integral protein, p7. The nonstructural (NS) proteins include NS2, NS3, NS4a, NS4b, NS5a, and NS5b.

The HCV core is a basic protein with RNA-binding properties. It is believed to form the nucleocapsid in association with the viral RNA. Additionally, the core protein has been shown to modulate the transcription and translation of several cellular genes (68) and have oncogenic effects in cell cultures and transgenic mice. E1 and E2 envelope proteins coat the virus. Comparison of HCV sequences has shown two hypervariable regions (HVRs) within E2, HVR1 and HVR2. Mutations in HVR1 emerge rapidly under selective host immune pressure (69). E2 may elicit the production of virus-neutralizing antibodies, resulting in immune selection of HCV genomes with escape mutations in HVR1 (69). In addition, E2 has been shown to be involved in the binding process of HCV to the host cell. The function of p7 is partly unknown; however, it may be necessary for HCV replication and may act as a viroporin (70,71). NS2 has protease activity. Together with the N terminus of NS3, NS2 forms an NS2/NS3 protease responsible for autocatalytic cleavage at the NS2/NS3 junction (72). NS3 has both protease and helicase activities. NS4a essentially functions as a cofactor for NS3. Moreover, HCV NS3/4a serine protease has been shown to block the action of IFN regulatory factor-3,

a key cellular antiviral molecule (73). The function of NS4b is unknown, while NS5a plays a role in modulating the effect of IFN on the virus. A region in NS5a has been identified as an IFN-sensitive determining region (ISDR) where a close association is demonstrated between mutations in this region and sensitivity to IFN therapy (74). In addition, NS5a has been shown to be a potent inhibitor of the IFN-induced protein kinase PKR, an antiviral molecule (74). NS5b possesses RNA-dependent RNA polymerase activity and is essential for RNA synthesis and viral replication. The details of how viral RNA-dependent RNA polymerase initiates RNA synthesis remain poorly understood.





▪ **Figure 26.4** Schematic representation of the HCV genome. Unshaded regions indicate the major structural proteins: Core *(c)*, envelope (E1 and E2), and p7. The shaded region indicates the nonstructural proteins NS2, NS3, NS4a/NS4b, and NS5a/NS5b. At the 5′ untranslated region (5′-UTR) resides the internal ribosomal entry site (IRES).

Hepatitis C Virus Life Cycle

The mechanism by which HCV enters target cells is still unknown, but it involves the HCV glycoproteins E1 and E2. Host molecules observed to play a role in HCV cell entry include dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), and liver/lymph node-specific intercellular adhesion molecule 3-grabbing integrin (L-SIGN) (75,76). In particular, CD81 has been shown to function as a postattachment entry coreceptor, while other unknown cellular factors act in concert with CD81 to mediate HCV binding and entry into hepatocytes (77). Similarly, low-density lipoprotein receptor (LDLR) has been shown in vitro to act as entry point for HCV (78,79).

The virus selectively fuses with liver cell plasma membranes. Once fusion of the viral lipid coat and host plasma membrane is complete, the viral core enters the host cell (Fig. 26.5). The HCV genome can be directly read by the host's ribosomes. During translation the ribosomes produce a polyprotein that is then processed into the 10 HCV proteins. The enzymes NS3 serine protease and NS2/NS3 protease cleave the polyprotein. When adequate RNA transcriptase is produced, an antisense version of HCV RNA is made to serve as a template for RNA replication. The enzyme NS3 helicase unwinds the RNA during replication, and NS5b, or the HCV RNA–dependent RNA polymerase, catalyzes RNA synthesis. The newly produced RNA and processed proteins assemble to form viruses that travel to the inside portion of the plasma membrane and then exit the host cell.

Recently, a full-length genotype 2a HCV genome that replicates and produces infectious virus particles in cell culture has been described (80).

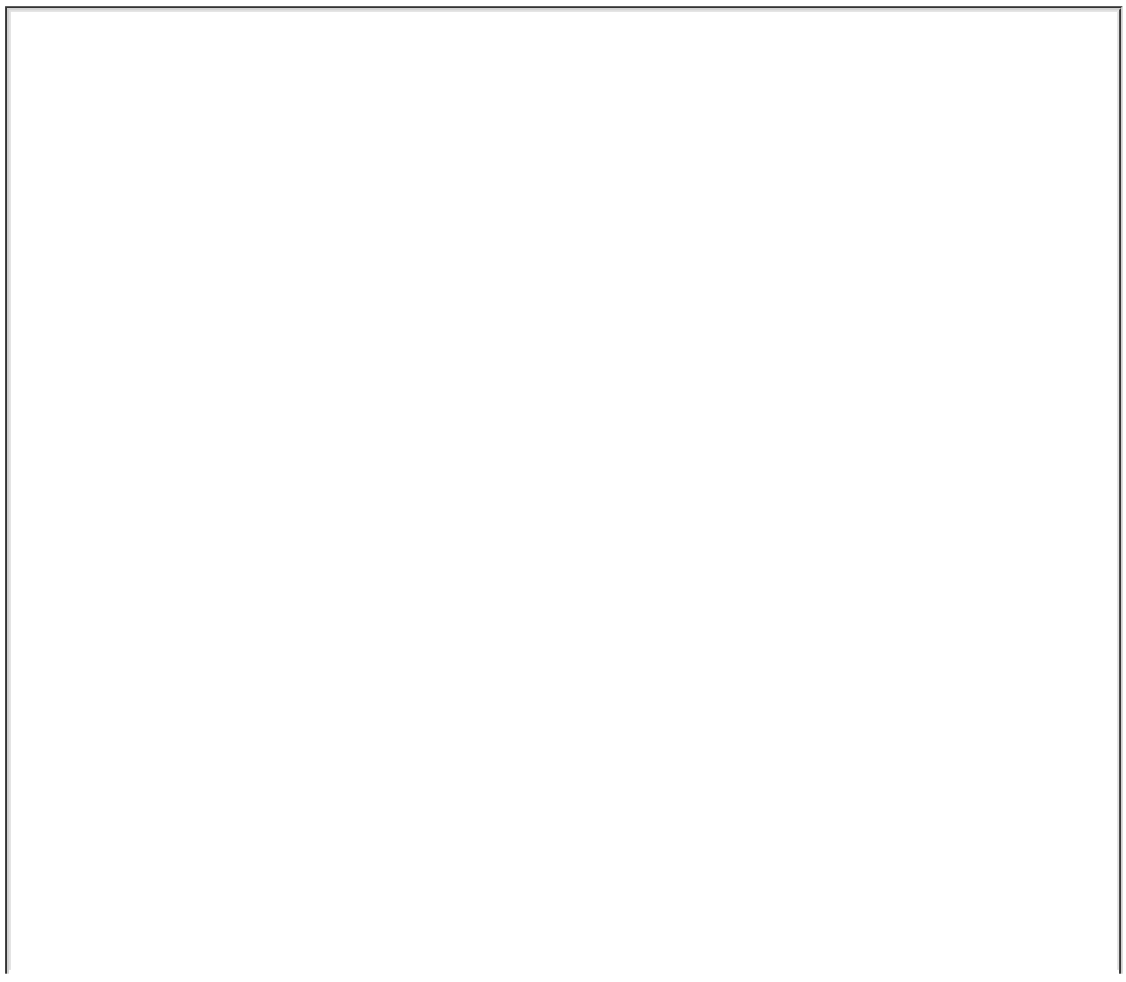
This system promises to lay the foundation for future in vitro studies to examine and shed further light on the life cycle of the virus.

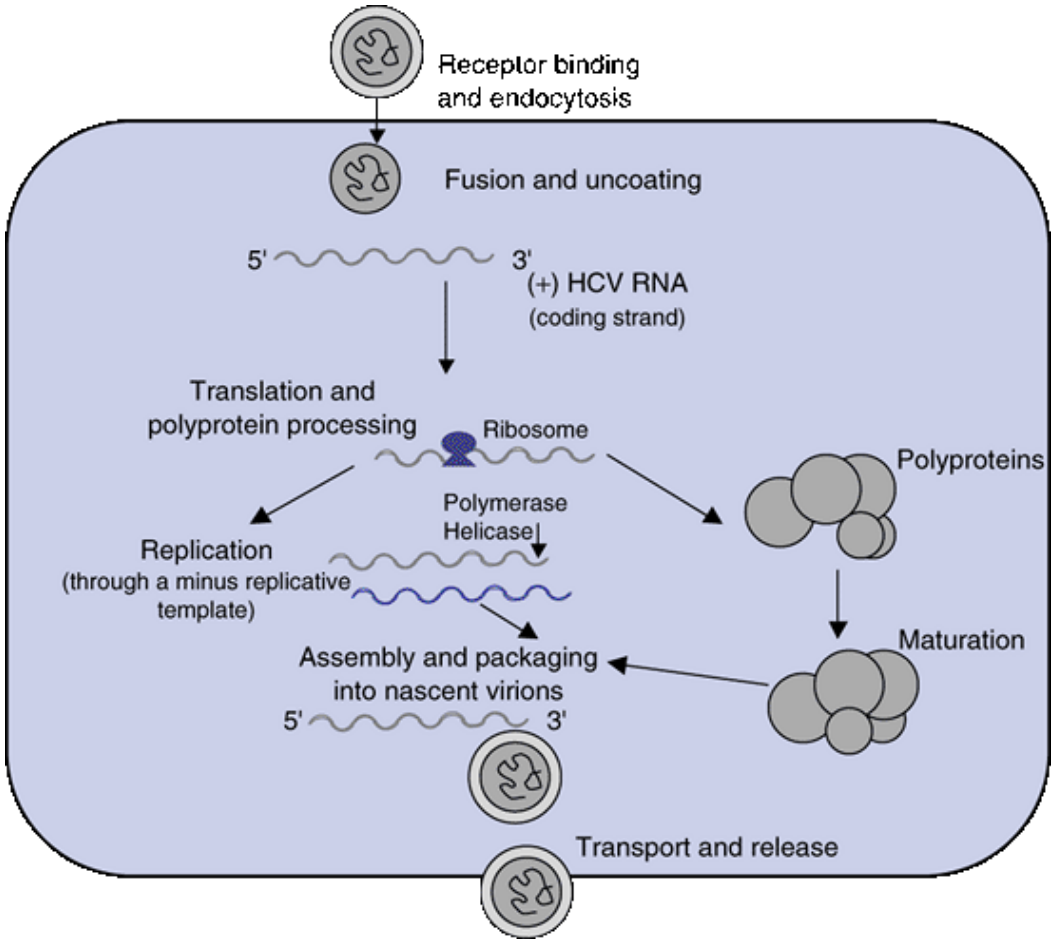
Natural History of Hepatitis C Virus Infection

Acute HCV infection is usually asymptomatic, and therefore, the timing of disease onset is usually assumed on the basis of exposure to specific risk factors. Early studies suggested that most acutely infected patients (80% to 90%) developed chronic HCV infection. However, certain more recent studies with longer follow-up indicate that only approximately 50% of acutely infected patients progress to chronicity (81,82,83). Progression of chronic HCV infection is usually slow, with advanced disease developing only 10 to 30 years or even longer after infection (84,85). The degree of fibrosis on the initial liver biopsy is the main determinant of progression to cirrhosis and the development of subsequent liver complications (85,86). Other factors associated with increased progression to fibrosis include male gender, older age at initial infection, excess alcohol, coinfection with HBV or HIV, and the presence of hepatic steatosis.

In patients with cirrhosis, the 5-year risk of decompensation is 18%, with a 5-year survival probability around 50% in those who decompensate (87,88). Moreover, in patients with cirrhosis, the 5-year risk of developing HCC is around 7% and 18% in those

with compensated and decompensated liver disease, respectively (88).





▪ **Figure 26.5** Schematic representation of the life cycle of hepatitis C virus (HCV). The main steps in the cycle are viral entry and uncoating, translation, replication, and packaging (see text for details). HCV RNA, hepatitis C virus ribonucleic acid.

Immunopathogenesis of Hepatitis C Virus Infection

It is unclear why a substantial proportion of patients with HCV fail to permanently eradicate the virus after acute infection. Several arms of the immune system are important in orchestrating viral clearance. Animal studies indicate that sustained or even transient clearance of HCV is clearly associated with an intrahepatic immune response characterized by increased expression of IFN-γ–inducible genes (89). The upregulation of these genes has been observed to occur as early as 2 days after infection, reflecting activation of the innate immune response and its crucial role in viral clearance (89). Similarly, after acute infection, high and persistent titers of neutralizing antibodies have been detected in patients in whom the virus is eradicated, in contrast to a low and nonsustained neutralizing antibody response in those with persistent infection (90). These neutralizing antibodies are however variant specific and, therefore, do not

provide protection against reinfection with other viral variants (91,92).

The cellular immune response also plays a major role in eradicating HCV infection. Multispecific vigorous intrahepatic CD4 and CTL responses are detected in subjects who have successfully cleared an acute HCV infection (93,94). The CD4 response associated with viral clearance is dominated by a T_H1 cytokine profile with increased IFN-γ production. This T_H1 cytokine response has been shown to coincide with a decline in the levels of viremia in acutely infected chimpanzees (89,95). The CD4 response is also essential for the functional maturation of CTL cells. Successful viral elimination has been shown to be associated with the induction of an early intrahepatic CTL population that possesses cytolytic and noncytolytic activities (95). The CTL cytolytic response is associated with acute hepatitis, and the vigor of the response parallels serum ALT levels and the extent of liver injury (96,97). However, HCV clearance and resolution of infection coincides with the appearance of CTL-induced cytokines (mainly IFN-γ) (96,97).

In addition to the immune response, nonimmunologic genes, in particular, genes associated with lipid metabolism, have been recently recognized to influence HCV clearance (89). Genes related to the serum response element–binding protein signaling pathway appeared to enhance viral replication (89).

Liver injury in patients with chronic infection is primarily immune mediated; however, a cytopathic effect is apparent in association with HCV genotype 3 infection and in immunosuppressed patients. In chronic liver injury, the HCV-specific CTL immune

response may target not only virally infected cells but also uninfected bystander hepatocytes through the release of soluble proapoptotic mediators such as Fas-ligand and soluble TNF-α (98,99). In addition, antigen-nonspecific recruitment of T cells occurs at the site of hepatic damage in chronic hepatitis, further contributing to the extent of liver injury (100).

Nonimmunologic factors such as steatosis and insulin resistance have also been linked to the progression of liver injury in HCV-infected patients. In particular, in patients infected with HCV genotype 3, steatosis and insulin resistance are primarily viral mediated (cytopathic), whereas host factors, principally those associated with insulin resistance and its clinical attributes, are responsible for the development of steatosis in genotype 1–infected patients. The molecular mechanisms responsible for these observations have not been elucidated (101,102).

Treatment of Hepatitis C Virus Infection

In the minority of patients presenting with acute HCV, increasing data indicate that IFN treatment is effective in preventing the progression to chronicity. In particular, a 6-month course of pegylated IFN-α in acute HCV infection has been shown to achieve sustained viral clearance rates (SVRs) of 95% 6 months after treatment (103). These patients remain virus free in the long term (104). There are still, however, several unresolved issues

concerning acute HCV treatment including the timing and optimal duration of therapy and identifying those most likely to benefit from therapy, in particular, intravenous drug users.

Incremental advances have also been made in relation to therapy for chronic HCV infection. Presently, the combination of pegylated IFN-α and ribavirin is the standard therapy for chronic HCV infection. Response and duration of treatment (24 to 48 weeks) is dependent on the infecting genotype, with 76% to 80% of patients with genotypes 2 and 3, but only approximately 40% to 45% with genotypes 1 and 4, achieving an SVR 6 months after completing therapy (105,106). However, SVR after pegylated IFN-α and ribavirin therapy are approximately 10% lower for patients with bridging fibrosis or cirrhosis (105,106), and hence the benefits of maintenance pegylated IFN-α in this group of patients is under evaluation. The benefits of combination therapy also extends to other "difficult-to-treat groups" such as those with recurrent HCV infection after liver transplantation or those with HCV/HIV coinfection (107). In all these groups, however, drug-related adverse events are common, requiring dose reduction and the frequent use of supportive growth factors.

Hepatitis Delta Virus

Epidemiology

HDV has a worldwide distribution but its infectivity relies on HBV envelope proteins (108). Therefore, it has been estimated that around 5% of global HBsAg carriers are also infected with HDV. However, the geographic distribution of HDV and HBV differ, with high HDV prevalence rates in Mediterranean countries, where interfamilial transmission predominates (109). In contrast, HDV infection is uncommon in the United States and northern Europe, where transmission is usually through intravenous drug use (110). Other modes of HDV transmission are similar to HBV spread and include hemodialysis, and sexual and, uncommonly, vertical transmission (110). Given the close link between HDV and HBV, strategies employed to control HBV in recent years, in particular vaccination, have resulted in a significant decrease in the incidence of HDV infection.

Hepatitis Delta Virus Genome and Proteins

The HDV virion is a spherical particle with a diameter of 36 to 43 nm. Its envelope protein is supplied by the HBsAg and contains the HDV genome. The HDV genome is a 1.7-kb single-stranded circular RNA, which encodes a single structural protein, the HDAg, the only gene product of the virus (111). There are two isoforms of the HDsAg, a small form (S-HDAg, 195 amino acids) required for HBV DNA replication and a large form (L-HDAg, 214 amino acids) required for virion assembly and inhibition of replication (112,113).

The genome sequence of HDV is nearly self-complementary such that about 70% of the nucleotides are base paired on a rod-like double-stranded RNA structure (genomic and antigenomic strands) (111). Moreover, its genomic and antigenomic RNA have the capacity to act as ribozymes directing self-

cleavage and self-ligation during HDV replication.

Hepatitis Delta Virus Life Cycle

HDV enters the cells through a cellular receptor shared by HBV; however, the identity of this receptor is still unknown. HDV is then transferred to the nucleus, where replication occurs. The HDV genome utilizes host RNA polymerase II to direct RNA-dependent RNA synthesis. Three forms of RNA gather in the infected hepatocyte during HDV replication—genomic RNA, its complement antigenomic RNA, and an antigenomic-sense mRNA. The antigenomic mRNA encodes the S-HDAg, which promotes the transcription/replication process (114,115). HDV replication occurs in the

nucleus by a double–rolling circle mechanism (116); the ribozymes are central to this mechanism. According to this mechanism, the circular genomic RNA serves as a template for an antigenomic RNA intermediate. This RNA intermediate is processed by autocatalytic cleavage at the ribozyme sites to yield monomeric linear RNA. In the final step, the linear RNA is ligated by HDV ribozyme and a host RNA ligase enzyme into an antigenomic, circular RNA. The circular antigenomic RNA subsequently serves as a template for a second round of rolling-circle replication. During replication, a unique event called *editing* occurs, allowing the generation of L-HDAg from S-HDAg. Up to 30% of the S-HDAg is edited by a host RNA–modifying RNA enzyme called *adenosine deaminase*. This changes the UAG stop codon of S-HDAg to a tryptophan (UGG) codon, resulting in the extension of the C terminal of the S-HDAg by 19 amino acids to generate the L-HDAg (117). Subsequently, L-HDAg inhibits viral replication and triggers the assembly and copackaging of viral particles (118). The editing process is therefore essential for achieving the balance between the conflicting but essential functions of the S-HDAg (RNA replication) and the L-HDAg (RNA inhibition). Virion assembly is triggered by an interaction between L-HDAg and HBsAg. The mechanisms by which this interaction occurs have not yet been fully elucidated.

Natural History of Hepatitis Delta Virus Infection

HDV infection can occur in three settings: An individual who becomes *coinfected* simultaneously with HDV and HBV, a chronic HBV-infected patient who develops *superinfection* with HDV, and finally, reinfection following liver transplantation. The clinical expression and outcome of each of these modes of infection are somewhat different.

In patients who develop *coinfection*, acute delta infection (and similarly that of HBV) is often self-limiting and progression to chronic liver disease is uncommon, occurring in around 2% of cases. The clinical spectrum of *coinfection* may range from asymptomatic disease to fulminant hepatitis.

HDV *superinfection* primarily affects intravenous drug users and chronic HBV carriers in developing countries. The preexisting HBV provides the substrate for rapid replication and spread of HDV, hence *superinfection*

usually results in chronic HDV infection or, alternatively, may cause severe fulminant hepatitis. Patients chronically infected with both viruses have progressive liver disease, with most developing cirrhosis. Moreover, the risk of HCC is increased threefold in patients with cirrhosis and dual HBV/HDV infection (41).

After liver transplantation, HDV reinfects the graft soon after surgery, resulting in subclinical infection that becomes symptomatic only if HBV reactivates, producing a florid hepatitis that does not usually respond to therapy (119).

Immunopathogenesis of Hepatitis Delta Virus Infection

It is not clear whether the enhanced disease severity observed from dual HBV/HDV infection is due to the immune response against viruses, a direct HDV cytopathic effect on hepatocytes, or a combination of these processes. The observation that the extent of portal inflammation correlates with the number of HDAg-positive cells suggests an immunologic mechanism contributing to the liver lesion. Investigations into a potential cytopathic role for the delta virus, however, have generally yielded conflicting results. A cytopathic effect has been suggested because HDAg produces significant cell death in transfected cell lines (120). Additionally, microvesicular steatosis has been frequently observed in association with epidemics of HDV infection and in patients undergoing transplantation who exhibit the delta virus (121). In contrast to these observations, transgenic mice expressing HDAg develop no hepatic damage (122). Similarly, in patients undergoing transplantation who are reinfected with HDV alone, hepatitis does not ensue until the reappearance of HBV antigens in the graft (121,123).

Treatment of Hepatitis Delta Virus Infection

Treatment of chronic hepatitis due to infection with HDV/HBV is unsatisfactory, and IFN-α is the only agent found to have any effect. To achieve therapeutic benefit, patients require treatment with a high dose of IFN-α (5 to 9 MU three times weekly) for at least 12 months (124). This regimen has been shown to result in sustained normalization of ALT levels and improved liver histology in 50% of treated patients. A recent 14-year long-term follow-up report of patients who received this treatment showed that responders also achieved clinical and survival benefits (125). Some long-term responders cleared HDV RNA and, eventually, HBV (125). Similar long-term improvements are observed in hepatic necroinflammatory activity and fibrosis (125). Unfortunately, IFN-related side effects are common in this group, particularly psychiatric problems, often leading to discontinuation of therapy (126,127).

Results with other antiviral agents have been disappointing. Lamivudine alone or in combination with IFN was not associated with any histologic or biochemical improvement (128,129). Hence, in contrast to treatment of HBV infection, available evidence does not support the use of nucleoside

analogs for the treatment of chronic HDV infection.

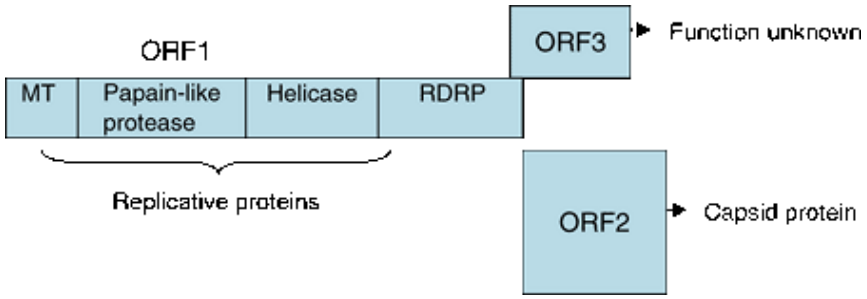
Hepatitis E Virus

Epidemiology

Hepatitis E is a worldwide public health problem and particularly causes acute hepatitis in adults through most of Asia, the Middle East, and northern Africa (130,131). In India, acute HEV is responsible for 60% of patients presenting with fulminant hepatitis. Moreover, cyclic outbreaks of HEV have occurred in India, Iraq, Sudan, Mexico, and parts of Asia. Sporadic cases of acute HEV have occurred in both South America and Europe. In contrast, cases of HEV are rare in the United States and are primarily linked to travel to endemic regions (130). HEV is enterically transmitted, and epidemics are usually linked to contaminated water supplies. Parenteral transmission may occur in endemic areas (132) and vertical (mother-to-infant) transmission has also been reported (133). In contrast to HAV, which predominantly affects children and spreads secondarily in 15% of patients, HEV mainly affects young adults and has only a low rate of secondary spread (2%).

Hepatitis E Virus Genome, Proteins, and Life Cycle

HEV is a small spherical nonenveloped RNA virus of approximately 30 nm diameter. Although its genomic structure shares some morphologic similarities to the Caliciviridae and Picornaviridae families, HEV remains unclassified at this time. The viral genome consists of a 7.5-kb plus-strand RNA with a 5′ capped end and a polyadenylated 3′ end (134). The genome (Fig. 26.6) contains three distinct ORFs (ORF1, ORF2, and ORF3) (134). ORF1 extends approximately 5 kb from the 5′ end and encodes polyprotein-containing sequence motifs of methyltransferase, papain-like protease, RNA helicase, and an RNA-dependent RNA polymerase. ORF2 begins 37 bp downstream of ORF1 and extends approximately 2 kb to the termination codon. The sequence of ORF2 suggests that it encodes the gene for the capsid protein. Moreover, ORF2 contains important epitopes that can induce neutralizing antibodies and has therefore been the focus of vaccine development (135). Finally, ORF3 partially overlaps ORF1 and ORF2 and encompasses only 369 bp. It encodes a protein that may play a role in viral infectivity (136). The genomic organization of the virus is consistent with the 5′-end encoding nonstructural and the 3′-end encoding structural viral gene(s) (134).



▪ **Figure 26.6** The hepatitis E genome—the genome is a capped, single-stranded positive-sense ribonucleic acid (RNA) molecule. It contains three distinct open reading frames (ORFs). ORF1 encodes replicative proteins including methyltransferase (MT), papain-like protease, helicase, and RNA-dependent RNA polymerase (RDRP). ORF2 encodes the capsid protein. The small overlapping ORF3 encodes a protein of unknown function.

The virus cannot be grown reliably and reproducibly in cell cultures and, therefore, the replication cycle is poorly understood. It is assumed that the virus attaches to receptor sites on hepatocytes and, possibly, biliary and intestinal cells. After uncoating, the genome is translated to produce ORF, subsequently cleaved by cellular proteases. It is postulated that replicative negative strand RNA intermediates are synthesized and then act a template for the synthesis of genomic and subgenomic positive strands (137). The mechanisms involved in viral assembly and transport are not known.

Natural History and Immunopathogenesis

HEV causes an acute, self-limiting illness with no chronic sequelae. Serologic follow-up studies of infected patients confirmed that antibodies to HEV provide long-term protection against the disease (138). Acute HEV infection, however, results in a high mortality among pregnant women, particularly during the third trimester, with case fatality rates ranging from 5% to 25% (133).

The mechanisms involved in hepatocyte destruction during acute HEV remain unknown. In HEV-infected cynomolgus macaques, the lymphocytes involved in the liver lesion have been found to be positive for a cytotoxic/suppressor immunophenotype (139). Additionally, hepatocyte culture of the cynomolgus monkey inoculated with a transmissible stool extract of HEV did not show any cytopathic change (139). Therefore,

the data from this single study suggest an immune-mediated rather than a

direct cytopathic mechanism for the liver injury in HEV infection.

Treatment of Hepatitis E Virus Infection

There is no specific treatment for acute HEV infection. Public health strategies to improve sanitation and handling of food and water are currently the best prophylaxis against the disease (131). Vaccine development strategies are under way, particularly examining the ORF2-encoded HEV proteins.

Hepatitis G Virus

Epidemiology

The HGV and the GB-C virus are two variants of the same virus and are therefore often referred to in the literature as GBV-C/HGV; in this review we use the term HGV (140). A direct association with liver pathology, however, is still lacking, and it is therefore unclear whether HGV is a hepatotropic virus. HGV is distributed worldwide, and carriage of the virus has been documented among healthy individuals and different patient groups (141). Between 1% and 4% of healthy blood donors have detectable serum HGV RNA (141,142). The highest prevalence rates are in Thailand, Vietnam, and Africa while China, Japan, and the United States have low prevalence rates (143,144). The virus is frequently found in populations at risk for blood-borne or sexually transmitted viruses, and there is also evidence of transmission vertically from mother to infant (145,146). Because of shared modes of transmission, many patients with HGV are coinfected with other blood-borne viruses such as HCV, HBV, and HIV (147).

The Hepatitis G Virus Genome, Proteins, and Life Cycle

GBV-C/HGV is a single-stranded positive-sense RNA virus, which is a member of the Flaviviridae. It has close sequence homology and genomic organization with HCV (148). The HGV genome is preceded by a 5′ UTR, which is followed by a long ORF (2,842 to 2,933 amino acids), terminating in a 3′ UTR. The structural proteins are E1 and E2, while the nonstructural proteins are NS2, NS3, NS4a, NS4b, NS5a, and NS5b. However, unlike HCV, the coding region for the core gene remains unknown. Also, the E genes are highly conserved among the various isolates of HGV, and therefore, there is no hypervariable region in the HGV genome (148). The 5′ UTR contains an IRES that is capable of directing cap-independent translation of the polyprotein (149). NS2 has a zinc-dependent protease, and the NS3 gene has a helicase motif and is likely to be essential for replication. NS5b gene encodes an RNA-dependent RNA polymerase (148), while the function of the other NS4 proteins is unknown. The exact site of HGV replication is unknown, although it appears that the virus replicates within lymphocytes and, perhaps, hepatocytes (150,151).

Natural History and Immunopathogenesis of

Hepatitis G Virus

The question of whether HGV contributes to liver disease either alone or in association with other chronic hepatitis virus infections has been extensively investigated with conflicting results, ranging from implicating the virus in cases of fulminant hepatitis to negating any role for the virus in liver disease. The prevalence rates of HGV associated with fulminant hepatitis has been reported to range from 5% to 15% (152,153). However, other studies sequentially examining the blood of patients with fulminant hepatitis concluded that the presence of HGV in them was accidental and coincided with the receipt of contaminated blood products in the course of therapy (154,155,156). Most individuals with HGV infection alone appear to have an asymptomatic course; many eventually clear the virus, developing antibodies to the E2 envelope glycoprotein that also protect against reinfection (144,146). The incidence of HGV among patients with HCV infection varies from 11% to 24% (157). However, HGV appears not to influence the clinical or virologic course of HCV infection nor does it have an impact on the response to IFN therapy (158,159). Whether HGV contributes to the development of HCC is still controversial, although it appears unlikely (160,161).

More recently, coinfection with HGV and HIV has been shown to improve mortality and morbidity for the HIV-infected individuals and slow the progression to acquired immunodeficiency syndrome (AIDS) (162,163).

Herpesviruses

Several other viruses, which are not primarily hepatotropic, may also cause hepatitis. These are usually herpesviruses, a family of double-strand DNA viruses, which can cause either chronic infections or enter a period of viral latency. So far, eight human herpesviruses have been identified: Herpes simplex virus 1 and 2 (HSV1 and HSV2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), and human herpesviruses 6, 7, and 8.

HSV1 and HSV2 commonly produce self-limiting vesicular or ulcerative lesions primarily involving the orofacial (HSV1) or the genital areas (HSV2). In the immunocompromised host or neonates, generalized infections including hepatic involvement may occur. Neonatal disseminated HSV infection can cause rapidly progressive multiple organ failure, with an 85% mortality rate if left untreated (164). In adults, the risk factors for the development of HSV hepatitis include third trimester pregnancy, an underlying immune-modifying disease such as malignancy, or the use of immunosuppressive medications particularly in solid organ transplant recipients (165,166). The clinical presentation of HSV hepatitis is nonspecific and includes fever, abdominal pain, and flu-like symptoms. Up to 30% of patients may not have detectable mucocutaneous lesions (167). Most of those who progress to fulminant hepatitis display a characteristic pattern of liver test abnormalities, the so-called anicteric hepatitis, with minimal elevation of serum bilirubin levels in the presence of marked elevations in the levels of serum transaminases. Progressive liver failure

often ensues. Disseminated HSV infection is treated with high-dose intravenous acyclovir (5 to 10 mg/kg three times daily) (168).

VZV is primarily a self-limiting illness of childhood; however, reactivation of VZV occurs in the setting of advancing age or reduced immunity (169). Clinical hepatitis occurs in the context of disseminated VZV and can rarely be fatal. VZV is best managed with high-dose intravenous acyclovir (168).

Latent CMV infections are often reactivated in the setting of immunocompromised hosts. AIDS patients, with low CD4 count are particularly at risk for symptomatic CMV infection. CMV is also a major pathogen in patients undergoing transplantation. CMV infection has been documented in 23% to 85% of liver transplant recipients; half will have symptomatic disease. Moreover, in recurrent HCV infection after liver transplantation, CMV reactivation increases the risk of hepatic fibrosis and allograft dysfunction (170). In immunocompromised hosts, typical liver lesions include CMV inclusion bodies in hepatocytes, vascular endothelium, and particularly, biliary epithelium. Ganciclovir is often used to treat CMV hepatitis; however, drug resistance may emerge and in these cases, foscarnet is the alternative agent of choice (171).

Infectious mononucleosis, as a result of EBV infection, is usually associated with only mild hepatitis. Most cases resolve spontaneously, although acute hepatic failure in immunocompetent patients has occasionally occurred. In addition, primary or secondary EBV infection has been linked to an increased risk of lymphoproliferative disorders after solid organ transplantation (172).

Human herpesvirus (HHV)-6 and HHV-7 are novel members of the betaherpesvirus family. The clinical effect of HHV-6 and HHV-7 reactivation in recipients of liver transplants who are also coinfected with CMV is now being recognized (173). In this regard, primary or secondary HHV-6 or HHV-7 infection after liver transplantation has been observed to be associated with predisposition to invasive and symptomatic CMV infection (173,174).

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Chapter 27

Hepatitis A

Maria H. Sjögren

Key Concepts

- Hepatitis A is a worldwide infection whose epidemiology is changing as countries improve water sanitation and use other preventive methods such as immunization.
- The precise mechanism of hepatic uptake in human liver is unknown and the cellular receptor for hepatitis A virus (HAV) has not been definitively identified; once infection occurs, HAV is distributed throughout the liver.
- Infection with HAV does not result in chronic disease. Rarely, it can have a prolonged course or a relapsing course and occasionally, profound cholestasis can occur. Mortality rate is low in previously healthy persons. Morbidity can be significant in adults and older children.
- Acute hepatitis A is clinically indistinguishable from other forms of viral hepatitis. A diagnosis of acute hepatitis A requires demonstration of immunoglobulin M (IgM) anti-HAV in serum.
- Original recommendations in 1999 by the Advisory Committee on Immunization Practices (ACIP) had the overall strategy to immunize populations at risk. In 2005, the ACIP recommended that all children between 1 and 2 years of age receive the hepatitis A vaccine.

Experimental work in humans led to the clinical recognition that viruses were etiologic agents of hepatitis A ("infectious hepatitis") and hepatitis B ("serum hepatitis") (1,2). Later, the existence of two hepatitis viruses was demonstrated: Hepatitis A virus (HAV) and hepatitis B virus (HBV) (3). HAV was first characterized in 1973, when scientists detected the virus in stools from human volunteers who were infected with HAV (4). The ensuing development of sensitive and

specific serologic assays for the diagnosis of HAV infection and the isolation of HAV in cell culture (5) were important advances that permitted the understanding of the epidemiology of HAV infection and, ultimately, control of the disease.

Virology

In 1982, HAV was classified as an enterovirus type 72 belonging to the Picornaviridae family. Subsequent determination of the sequence of HAV nucleotides and amino acids led to questioning of this classification, and a new genus, hepatovirus, was created for HAV (6).

HAV has an icosahedral shape and is a nonenveloped virus. It measures 27 to 28 nm in diameter, has a buoyant density of 1.33 to 1.34 g/cm (3) in cesium chloride, and has a sedimentation coefficient of 156 to 160S by ultracentrifugation. HAV survives exposure to ether and an acid environment at pH 3. It also survives heat exposure at 60˚ C for 60 minutes but is inactivated at 85˚C for 1 minute. HAV is capable of surviving in seawater (4% survival rate), dried feces at room temperature for 4 weeks (17% survival), or live oysters for 5 days (12% survival) (7).

Only one serotype of HAV is known, and there is no antigenic cross-reactivity with the hepatitis B, C, D, E, or G agents. The HAV genome consists of a positive-sense ribonucleic acid (RNA) that is 7.48 kb long, single stranded, and linear. HAV RNA has a sedimentation coefficient of 32 to 33S and a molecular weight of

2.8×10^4. The HAV RNA has a long open reading frame of 6,681 nucleotides and is covalently linked to a 5’ terminal protein and a 3’ terminal polyadenosine tract.

The onset of HAV replication in cell culture systems takes from weeks to months. Primate cells, including African green monkey kidney cells, primary human fibroblasts, human diploid cells (MRC-5), and fetal rhesus kidney cells, are favored for the cultivation of HAV in vitro. The virus is not cytopathic, and persistent infection in the cell cultures is the rule. Two conditions control the outcome of HAV replication in cell culture (8). First, the genetic make-up of the virus is important; HAV strains mutate in distinct regions of the viral genome as they become cell culture adapted. The second condition is the metabolic activity of the host cell at the time of infection. Cells in culture, although infected simultaneously, initiate HAV replication in an asynchronous manner. This asynchronicity may be caused by differences in the metabolic activity of individual cells, but there is no definitive evidence of cell-cycle dependence of HAV replication (9).

An initial step in the life cycle of a virus is its attachment to a cell surface receptor. The location and function of these receptors

determine tissue tropism. Little is known about the mechanism of entry of HAV into cells. Some work has suggested that HAV could infect cells by a surrogate-receptor binding mechanism (by a nonspecified serum protein). HAV infectivity in tissue culture has been shown to require calcium and to be inhibited by the treatment of the cells with trypsin, phospholipases, and β-galactosidase (10). A surface glycoprotein, named HAVcr-1, on African green monkey kidney cells has been identified as a receptor for HAV. Blocking of HAVcr-1 with specific monoclonal antibodies prevents infection of otherwise susceptible cells. Experimental data suggest that HAVcr-1 not only serves as an attachment receptor but may also facilitate uncoating of HAV and its entry into hepatocytes (11).

Whatever the entry mechanism, once HAV enters a cell, the viral RNA is uncoated, cell host ribosomes bind to viral RNA, and polysomes are formed. HAV is translated into a large polyprotein of 2,227 amino acids. This polyprotein is organized into three regions: P1, P2, and P3. The P1 region encodes the structural proteins VP1, VP2, VP3, and a putative VP4. The P2 and P3 regions encode nonstructural proteins associated with viral replication.

The HAV RNA polymerase copies the plus RNA strand. The RNA transcript, in turn, is used for translation into proteins, which are used for assembly into mature virions. It appears that downregulation of HAV RNA synthesis occurs as defective HAV particles appear (12). In addition, a group of specific RNA-binding proteins have been observed during persistent infection (13). The origin and nature of these proteins is unknown, but they exert activity on the RNA template and are believed to play a regulatory role in the replication of HAV (14).

Numerous strains of HAV exist, with considerable nucleotide sequence variability (15% to 25% difference within the P1 region of the genome). Human HAV strains can be grouped into four different genotypes (I, II, III, and VII), whereas simian strains of HAV belong to genotypes IV, V, and VI (15). Despite the nucleotide sequence heterogeneity, the antigenic structure of human HAV is highly conserved among strains.

The HAV VP1/2A and 2C genes are thought to be responsible for viral virulence, on the basis of experiments in which recombinant HAV caused acute hepatitis in animals following the construction of 14 chimeric virus genomes from two infectious complementary deoxyribonucleic acid clones that encoded a virulent and an attenuated HAV isolate (HM175 strain) and the genotype and phenotype of each virus were compared (16).

Among the many strains of HAV, the HM175 and CR326 human HAV strains are important because they are used for the production of commercially available vaccines. Strain HM175 was isolated in 1978,

from the human feces of Australian patients in a small outbreak of hepatitis A. CR326 was isolated from Costa Rican patients infected with HAV. The nucleotide and amino acid sequences showed 95% identity between the two strains. Vaccines prepared from these strains are thought to provide protection against all relevant human strains of HAV.

Variations in the HAV genome are thought to play a role in the development of fulminant hepatic failure (FHF) during acute HAV infection. The 5′ untranslated region of the HAV genome was sequenced in serum samples from 84 patients with HAV infection, including 12 with FHF (17). The investigators observed relatively fewer nucleotide substitutions in the HAV genome of patients with FHF than in those without FHF ($P < 0.001$). The differences were most prominent between nucleotides 200 and 500, suggesting that nucleotide variation in the central portion of the 5′ untranslated region influence the clinical severity of HAV infection.

Epidemiology

Acute hepatitis A is a reportable infectious disease in the United States, with a rate of infection of 4/100,000 (18). In 2001, 10,616 cases of HAV infection were reported in the United States and in 2003, 7,653 cases of acute HAV infection were reported to the Center for Diseases Control and Prevention (CDC), the lowest number to date. Taking into consideration the underreporting of cases and the occurrence of asymptomatic infections, the true number of annual HAV infections

has been calculated to be 93,000 and 61,000 for both years, respectively (19). The highest rate of reported disease is among children aged 5 to 14 years; 25% of reported cases are among persons aged 20 years or less (19), but HAV infection can occur in any age-group.

The epidemiologic risk factors for HAV infection reported for the US population in 2002 were as follows: Unknown, 57%; sexual or household contact with a patient who has hepatitis A, 12%; international travel, 9%; male homosexual activity, 8%; injection drug use, 5%; child or employee in a day-care center, 1%; food or waterborne outbreak, 1%; contact with a day-care child or employee, 3%; and other contact with a patient who has hepatitis, 4% (20).

HAV infection generally follows one of three epidemiologic patterns (21). In countries where sanitary conditions are poor, most children are infected at an early age. Although earlier seroepidemiologic studies routinely showed that 100% of preschool children in these countries had detectable antibody to HAV (anti-HAV) in serum, presumably reflecting previous subclinical infection, subsequent studies have

shown that the average age of infection has increased rapidly to 5 years and above, when symptomatic infection is more likely. For example, 82% of 1,393 Bolivian school children were shown to have detectable anti-HAV, but when they were stratified into two groups according to family income, a significant difference was found between the groups: 95% of children from low-income families had detectable anti-HAV as against 56% of children from high-income families (22).

The second epidemiologic pattern is seen in industrialized countries, where the prevalence of HAV infection is low among children and young adults. In the United States, the prevalence of anti-HAV is approximately 10% in children but 37% in adults (23).

The third epidemiologic pattern is observed in closed or semiclosed communities, such as some isolated communities in the South Pacific, where HAV is capable (through epidemics) of infecting the entire population, which then becomes immune. Thereafter, newborns remain susceptible until the virus is reintroduced into the community.

Table 27.1. Detection of Hav and Infectivity of Human Secretions or Excretions

Secretion/excretion	Comment	References
Stool	Main source of infection. HAV is detectable during incubation period and for several weeks after the onset of disease. After the onset of symptoms, HAV is detectable in 45% and 11% of fecal specimens from first and second week, respectively, whereas HAV ribonucleic acid (by a polymerase chain reaction assay) is detectable for 4 to 5 mo.	(28,29)
Blood	Viremia is present	(30,31)

	during incubation period. Blood collected 3 and 11 days before the onset of symptoms caused posttransfusion infection in donors. Chronic viremia does not occur.	
Bile	HAV has been detected in the bile of chimpanzees infected with HAV.	(32)
Urine	HAV is detected in low titer during the viremic phase. A urine sample infected 1 of 12 subjects after oral inoculation. Urine contaminated with blood was also infectious.	(33,34)
Nasopharyngeal	Unknown in humans. HAV has been identified in the oropharynx of experimentally infected chimpanzees.	(35)
Semen, vaginal fluid	Uncertain. HAV may be detectable during viremic phase.	(36)

HAV, hepatitis A virus.

Whatever the epidemiologic pattern, the primary route of transmission of HAV is the fecal–oral route, by either person-to-person contact or ingestion of contaminated food or water. Although rare, transmission of HAV by a parenteral route has been documented following blood transfusion (24,25) or use of blood products (26). Cyclic outbreaks

among users of injection and noninjection drugs and among men who have sex with men (up to 10% may become infected in outbreak years) have been reported (27). Table 27.1 provides information about the detection of HAV and its infectivity in human body fluids (28,29,30,31,32,33,34,35,36).

Approximately 11% to 22% of patients with acute hepatitis A require hospitalization, with an average cost of $6,914 per patient (37). In one outbreak involving 43 persons, the total cost was approximately $800,000. On average, 27 workdays are lost per adult case of hepatitis A, with a total loss of 829,000 workdays/year in the United States. Combined direct and indirect costs associated with HAV infection in the United States totaled more than $200 million in 1989 and approximately $488.8 million in 1997 (27,37).

Pathogenesis

Once HAV is ingested and survives gastric acid, it traverses the small intestine mucosa and reaches the liver through the portal vein. The precise mechanism of hepatic uptake in humans is unknown (see preceding text). In an experimental model on African green monkey kidney cells (11), the putative cellular receptor for HAV has been identified as a surface glycoprotein.

Once the virus reaches the hepatocyte, it starts replicating in the cytoplasm, where it is seen on electron microscopy as a fine granular pattern, but it is not present in the nucleus. HAV is distributed throughout the liver. Although HAV antigen has been detected in other organs (lymph nodes, spleen, kidney), the virus appears to replicate exclusively in hepatocytes. Once the virus is mature, it reaches the systemic circulation through the hepatic sinusoids and is released into the biliary tree through the bile canaliculi, passed into the small intestine, and eventually excreted in the feces.

The pathogenesis of HAV-associated hepatocyte injury is not completely defined. The lack of injury to cells in cell culture systems suggests that HAV is not cytopathic. Immunologically mediated cell damage is more likely. The emergence of anti-HAV could result in hepatic necrosis during the immunologically mediated elimination of HAV.

Clinical Features

Infection with HAV does not result in chronic disease, but in an acute self-limited episode of hepatitis. Rarely, acute hepatitis A can have a prolonged or a relapsing course, and occasionally profound cholestasis can occur. Commonly, the incubation period is 2 to 4 weeks, rarely up to 6 weeks. The mortality rate is low in previously healthy persons.

Morbidity can be significant in adults and older children.

The clinical characteristics of cases of hepatitis A reported in 2002 were similar to those in previous years with a preponderance of cases in men of all age-groups. Overall, 72% of patients manifested jaundice, 25% required hospitalization, and 0.5% died (26). The need for hospitalization increased with age, from 5% among children older than 5 years of age to 34% among persons 60 years of age or older.

HAV infection usually presents in one of five different clinical patterns: (i) Asymptomatic without jaundice; (ii) symptomatic with jaundice and self-limited to approximately 8 weeks; (iii) cholestatic, with jaundice lasting 10 weeks or more; (iv) relapsing, with two or more bouts of acute HAV infection occurring over a 6- to 10-week period; and (v) FHF.

Children older than 2 years of age are usually asymptomatic; jaundice develops in only 20%, whereas symptoms develop in most children (80%) aged 5 years or older. A high rate of symptoms occurs in adolescents and adults. HAV infection with prolonged cholestasis is a rare variant but occasionally leads to invasive diagnostic procedures (inappropriately), because the diagnosis of acute hepatitis may not be readily accepted in patients with jaundice for several months, even in the presence of detectable anti-HAV of the immunoglobulin M (IgM) class (see the following sections) (38). A relapsing course is observed in approximately 10% of patients with acute hepatitis A. Shedding of HAV in stool has been documented during the relapse phase (39). This variant is benign, and the infection ultimately resolves (39). Neither the cholestatic variant nor the relapsing hepatitis A is associated with increased mortality. In all cases, treatment is symptomatic. Acute hepatitis A, unlike hepatitis E, is not associated with an increased mortality rate in pregnant women.

Prodromal symptoms in patients with acute hepatitis A include fatigue, weakness, anorexia, nausea, vomiting, and abdominal pain. Less common symptoms include fever, headache, arthralgias, myalgias, and diarrhea. Dark urine precedes other symptoms in approximately 90% of infected persons; this symptom occurs within 1 to 2 weeks of the onset of prodromal symptoms. Symptoms of hepatitis may last from a few days to 2 weeks and usually decrease with the onset of clinical jaundice. Right upper quadrant tenderness and mild liver enlargement are present on physical examination in 85% of patients; splenomegaly and cervical lymphadenopathy are each present in 15%. Complete clinical recovery is achieved in 60% of affected persons within 2 months and in almost everyone by 6 months. The overall prognosis of acute hepatitis A in otherwise healthy adults is excellent. Potentially fatal complications (e.g., FHF) develop in a few patients (see later sections).

Acute HAV infection must be differentiated from other causes of acute viral hepatitis, autoimmune hepatitis (AIH), and other causes of acute hepatitis by appropriate serologic testing (see Chapters 29 and 30). However, in some cases the diagnosis may be difficult to make because the patient may harbor more than one viral infection, such as chronic hepatitis B or chronic hepatitis C, with superimposed acute HAV infection.

Fulminant Hepatitis A

FHF caused by HAV is rarely seen in children, adolescents, or young adults. However, the case-fatality rate in people over 49 years of age with acute hepatitis A is reported to be 1.8%, compared with an overall rate of 0.3% in persons of all ages (39). Hepatic failure caused by hepatitis A becomes manifest in the first week of illness in approximately 55% of affected patients and during the first 4 weeks in 90%; FHF is rarely seen after 4 weeks (40).

The contribution of HAV to acute liver failure has been reported to be increased in populations classified as hyperendemic for HAV. In a report from India, where 276 patients with FHF were seen between 1994 and 1997, 10.6% of the cases among adults were caused by HAV. HAV had been responsible for only 3.5% of cases among 206 patients with FHF seen in the same community from 1978 to 1981 (41).

Certain populations have increased morbidity and a high risk of acute liver failure from HAV infection. Among these groups are the elderly (42) and persons with chronic liver disease. A 1998 report described the clinical outcome of 256 persons hospitalized for acute hepatitis A in Tennessee from January 1994 through December 1995 (43). On admission, 89% had experienced prolonged nausea or vomiting and 26% had a prolonged prothrombin time (>3 seconds); 39 had serious complications (19 were hepatobiliary in nature and 20 were extrahepatic complications), and 5 (2%) died. Morbidity and mortality correlated with age. Twenty-five percent of patients aged 40 and above had at least one complication, as compared with 11% of patients younger than 40 years of age ($P = 0.014$).

Although two reports since the late 1990s have described a decline in the number of cases of acute viral hepatitis among patients with FHF in the United States (44,45), this decline is attributable principally to the control of hepatitis B. The contribution of HAV infection to FHF has remained unchanged since the 1970s, despite the availability of highly efficacious vaccines.

Autoimmune Disease Following Acute Hepatitis A

Several viruses have been reported to trigger the onset of AIH. In rare cases, hepatitis A has been followed by the development of type 1 AIH. Genetic predisposition is thought to play a role (46,47).

Diagnosis

Acute hepatitis A is clinically indistinguishable from other forms of viral hepatitis. The diagnosis of infection is based on the detection of specific antibodies against HAV (anti-HAV) in serum. A diagnosis of acute hepatitis A requires demonstration of IgM anti-HAV in serum. The test is positive from the onset of symptoms (48) and usually remains positive for approximately 4 months (49). Some patients may have low levels of detectable IgM anti-HAV for more than 1 year after the initial infection (48). IgG anti-HAV is also detectable at the onset of the disease, remains present usually for life, and, following clinical recovery, is interpreted as a marker of previous HAV infection.

Testing for HAV RNA is limited to research laboratories. HAV RNA has been detected in serum, stool, and liver tissue. Viral RNA can be amplified by polymerase chain reaction (PCR) methodology (26). With a PCR assay, HAV RNA has been documented in human sera for up to 21 days after the onset of illness (50). The use of hepatitis C virus (HCV) RNA testing has been described in a report of 76 French patients with acute HAV infection seen between January 1987 and April 2000; 19 of them had FHF (51). Ten patients required liver transplantation, and one patient died while awaiting liver transplantation. The HAV RNA status was determined in 39 of the 50 patients in whom sera and clinical data were available, including the 19 with FHF. HAV RNA was detected in 36 of these 50 patients (72%). The likelihood that HAV RNA was undetectable was greater in patients with FHF than in those with nonfulminant hepatitis ($P < 0.02$). When HAV RNA was detectable, titers were lower in patients with encephalopathy than in patients with nonfulminant hepatitis (3.6 log vs. 4.4 log, $P = 0.02$). These data suggest that the detection of IgM anti-HAV and undetectable or low-titer HAV RNA in patients with severe acute hepatitis may signal an ominous prognosis and the need for early referral for liver transplantation. As in other studies, HAV genotype did not seem to play a role in the severity of clinical manifestations (52).

Prevention and Treatment

Recommendations concerning immunoprophylaxis of HAV were published in December 1999 by the Advisory Committee on Immunization Practices (ACIP) (27). The overall strategy is to protect persons from disease and to lower the incidence of HAV infection in the United States. The available vaccines are not licensed for use in children less than 1 year of age. Currently, all children should receive hepatitis A vaccine at age 1 year or older (52a), high-risk populations

are targeted for immunization; Table 27.2 lists these populations. Because children who reside in high-risk areas are targeted for vaccination, the overall rate of HAV infection has declined steadily, and in 2002, it was 3.1/100,000, the lowest rate yet recorded. The decline in rates has been greater among children than adults and in states where routine childhood vaccination is recommended,

suggesting that childhood vaccination has had a positive impact. Hepatitis rates declined 20-fold during the years 1997 to 2001 among American Indian and Alaska Native children where routine hepatitis A vaccine was implemented (53).

Table 27.2. Groups at High Risk of Hepatitis A Virus Infection

Healthy persons who travel to endemic areas, work in occupations for which the likelihood of exposure is high, are family members of infected patients, or adopt infants or children from endemic areas
Persons with chronic liver disease
Human immunodeficiency virus–positive patients
Men who have sex with men
Users of injection and noninjection illicit drugs
Persons with clotting factor disorders
Persons who live in communities with high or intermediate rates of HAV infection
Children who live in areas where the rate of HAV infection is at least twice the national average (≥20 cases/100,000 population)

HAV, hepatitis A virus.

However, a 2003 CDC analysis of hepatitis A vaccination coverage for children aged 24 to 35 months, who reside in the 11 states where the HAV vaccine is routinely recommended, showed that immunization ranged from 6.4% to 72.7% with an average of 50.9%, whereas immunization among children of the same age residing in the six states where HAV vaccination should be considered averaged 25% (range 0.6% to 32.3%). The analysis concluded that HAV immunization rates for children aged 24 to 35 months are lower than the overall rates for other children vaccines (54).

It is likely that universal immunization was not recommended in the United States because communities were considered to have high, intermediate, and low rates of hepatitis A and US government

surveillance data demonstrated that communities with high and intermediate rates were primarily responsible for an average of 50% of reported HAV cases each year (27). Hence, the recommendation was based on the concept that reducing the incidence of hepatitis A in states with high or intermediate average annual incidence of hepatitis A (Table 27.1) through routine vaccination of children would substantially reduce the incidence of the national disease. However, recent outbreaks in Georgia, Tennessee, and Pennsylvania where more than 600 symptomatic cases and three deaths were reported and thousands of exposed individuals required immediate passive immunization (55) seems to contradict the recommendation for immunization for high or intermediate rates of endemic HAV and it is likely that immunization directed to specific groups would not control the infection as efficiently as universal immunization would do. In October 2005, the CDC advisory committee recommended that all children in the United States receive the vaccine; therefore, it is expected that all children between the age of 1 and 2 years would have the HAV vaccine integrated into their childhood immunization programs (CDC press release October 28, 2005).

Table 27.3. Recommended Regimens for Hepatitis A Vaccination[a]

Vaccine	Age (y)	Dose	Volume (mL)	Dosing schedule (mo)
Havrix	2–18	720 EL.U.	0.5	0, 6–12
	>18	1440 EL.U.	1.0	0, 6–12
Vaqta	2–18	25 U	0.5	0, 6–18
	>18	50 U	1.0	0, 6–18
Twinrix	≥18	720 EL.U. hepatitis A virus	1.0	0, 1, 6

		20 µg hepatitis B virus		

[a]Vaccines are injected intramuscularly in the deltoid area. EL.U., enzyme-linked immunosorbent assay (ELISA) units.

There are no specific medications to treat acute hepatitis A; symptomatic treatment is the rule. Attention to sanitation and administration of serum Ig are the mainstays of preventing HAV infection. The availability of excellent HAV vaccines has rendered the use of Ig for pre-exposure prophylaxis unnecessary. When Ig is used for postexposure prophylaxis, it should be given within 2 weeks of exposure. In these cases, the recommended dose is 0.02 mL/kg by intramuscular injection. Although considered safe, Ig can cause fever, myalgias, and pain at the injection site. Postexposure prophylaxis with Ig can be accompanied safely with the initiation of active immunization with the vaccine (56).

The HAV vaccine was first licensed in the United States in 1995; two inactivated HAV vaccines are commercially available. Extensive use of the vaccines in clinical trials and postmarketing surveillance support the safety and efficacy of these products. Havrix is manufactured by SmithKline Biologicals, Rixensart, Belgium, and Vaqta, by Merck Sharp & Dohme, West Point, Pennsylvania. Both vaccines are derived from HAV grown in cell culture. The final products are purified and formalin inactivated; they contain alum as an adjuvant. The basic difference between the two commercially available vaccines is the HAV strain used for preparation. Havrix was prepared with the HM175 strain, whereas Vaqta was prepared with the CR326 strain (56,57); the difference is of little practical importance, because both vaccines are safe and immunogenic. The doses and schedule of immunization are shown in Table 27.3. Following vaccination with Havrix, anti-HAV is estimated to remain detectable in serum for approximately 20 years; immunity may last longer (58).

From the time the HAV vaccine was licensed in the United States through 1998, more than 6.5 million doses were administered, including 2.3 million pediatric doses. Worldwide, more than 65 million doses of HAV vaccine were administered through 1999. Among adults,

the most frequent local side effects were soreness at the injection site (56%), headache (14%), and malaise (7%). In children, the most frequent side effects were soreness at the injection site (15%),

feeding problems (8%), headache (4%), and injection site induration (4%).

In the United States, through 1998, the national Vaccine Adverse Event Reporting System received 247 reports of unexplained adverse events within 6 weeks of immunization. Approximately one third of these reports occurred with concurrent vaccinations and could not be attributed to the HAV vaccine. Thirteen events in children (0.6/100,000 doses distributed) and 85 events in adults (1.4/100,000 doses distributed) were considered serious. These events included neurologic, hematologic, and autoimmune syndromes. However, no reported serious event could be attributed definitively to the HAV vaccine, and the reported rates did not exceed the expected background rates. For example, the incidence of the Guillain-Barré syndrome ranges from 0.5 to 2.4 cases/100,000 person-years, and the 5 cases of Guillain-Barré syndrome among adult HAV vaccine recipients represented an incidence of 0.2 cases/100,000 person-years (27). Postmarketing reports have not shown a higher incidence rate in vaccine recipients.

A combined formulation of hepatitis A and B vaccines (Twinrix) is available and has an excellent record of efficacy and safety (59). The dosing schedule is shown in Table 27.3.

Immunization Against Hepatitis A Virus in Patients with Chronic Liver Disease

Persons with chronic liver disease are at increased risk of HAV-related morbidity and mortality if they acquire the infection. Therefore, pre-exposure prophylaxis with the HAV vaccine has been recommended for patients with chronic liver disease who are susceptible to HAV (60). This recommendation should be extended to pre- and post–liver transplant recipients, although the immunogenicity of the HAV vaccine is reduced in these persons (61,62).

An episode of acute hepatitis in a patient with underlying chronic liver disease poses the risk of considerable morbidity and mortality. Although the current guidelines recommend immunization against HAV for all patients with chronic liver disease (27), the results of several cost-effective analyses have been conflicting. A report published in 2000 found that saving the life of one patient with HCV infection by HAV vaccination would cost 23 million Canadian dollars (63). However, some of the assumptions in this report have been challenged (64). Two other studies of patients with chronic hepatitis C showed a decided benefit to immunization against HAV (65). The methods used in these studies were dissimilar, and some analyses may have been insensitive

to the incidence of HAV or may have underestimated the economic and societal costs of a case of FHF. Universal immunization against HAV during childhood, before the possible occurrence of chronic liver disease, offers the promise of preventing HAV infection (66).

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Although HAV infection does not produce chronic liver disease, morbidity and mortality can be considerable.

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Chapter 28

Hepatitis E

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Key Concepts

- Hepatitis E virus (HEV) infects humans by causing outbreaks of hepatitis or being the source of sporadic self-contained infections.
- Antibody to hepatitis E has been observed in many areas of the world; in the United States, 1% to 5% of healthy blood donors have detectable anti-HEV.
- Histopathologic features of HEV infection in the human liver include necroinflammatory processes and cholestatic hepatitis.
- The clinical features of HEV are difficult to differentiate from other viral hepatitis, the diagnosis needs to be suspected and corroborated with serologic tests.
- Case fatality rates among HEV-infected pregnant women have been reported to be between 15% and 25%.
- Development of a hepatitis E vaccine has successfully completed a phase III trial.

After hepatitis A and hepatitis B were diagnosed with the aid of accurate serologic testing, it became apparent that at least two non-A, non-B infectious agents existed, one similar to hepatitis B, mainly transmitted parenterally, and another similar to hepatitis A, transmitted by the fecal–oral route and without sequelae of chronic liver disease. In the 1980s, two seminal discoveries correctly identified the first one as hepatitis C (1) and the second one became known as *hepatitis E* (2).

Since then, hepatitis E has been recognized as the agent responsible for enterically transmitted non-A, non-B hepatitis. Research to understand the epidemiology, viral characteristics, and immunity against this viral agent was galvanized by the work of Balayan et al.

(2) and in recent years by cloning the virus (3), which allowed the development of assays not only to diagnose the infection but also to better understand its epidemiology and develop vaccine candidates.

Virology

Hepatitis E virus (HEV) was first visualized in 1983 when it was transmitted to a human volunteer and subsequently to an experimental animal model, thereby establishing its role as the etiologic agent of hepatitis E (2). HEV is a spherical nonenveloped virus 32 to 34 nm in size with spikes and indentations on its surface. It was recently classified into the separate genus *Hepatitis E–like viruses* (4). The genome of the virus is a single positive-stranded polyadenylated ribonucleic acid (RNA) of approximately 7.5/kb. It consists of three overlapping open reading frames (ORFs) and short untranslated regions at the 5′ and 3′ termini. ORF1 is located at the 5′ end and consists of the nonstructural genes while the 3′ end ORF2 represents one or more structural or capsid proteins. ORF2 contains important epitopes that can induce neutralizing antibodies

and is the prime genomic area selected for vaccine development (4). The function of ORF3 has not been elucidated.

Table 28.1. Hepatitis E Virus Genotypes (4)

Genotypes	Isolates
Genotype 1	Southeast Asia (e.g., Burmese, Indian) North and Central Asia (e.g., China, Pakistan, Kyrgyzstan, India)
	North Africa
Genotype 2	North America (Mexico)
Genotype 3	The United States
	(humans and swine)
Genotype 4	Subset of isolates from China and Taiwan

Heterogeneous	Europe, Argentina

Although there is no consensus on genotype classification, it is generally accepted that on the basis of viruses having nucleotide divergence of not more than 20% of the nucleotides in the ORF2 region, four major HEV genotypes exist (Table 28.1). In addition, genetically heterogeneous isolates from several European countries have been designated as new genotypes, but this concept is not widely accepted; similarly, two novel HEV genotypes have been described from Argentina (4). Despite the diversity of HEV genotype, it is accepted that HEV exists as a single serotype (5), a concept that has important implications in vaccine development because it makes the development of a broadly protective vaccine possible.

In 1990 the genome of HEV was cloned from infectious experimental animal bile. These experiments established that the clone ET1.1 represented a genuine portion of the HEV genome (3). Such advances permitted the development of sensitive and accurate assays that allow the diagnosis of the infection, the better understanding of its epidemiology, and development of candidate vaccines.

Epidemiology

HEV appears to infect humans by causing outbreaks of hepatitis or by being the source of sporadic self-contained infections. Although the hepatitis E outbreaks are newsworthy, anti-HEV has been detected in many areas of the world, including industrialized countries, where no defined epidemics have been reported, for example, in the United States 1% to 5% of healthy blood donors have detectable anti-HEV (6). The meaning of such a high prevalence is still a source of controversy. In addition to representing true HEV infection, some scientists attribute the high prevalence to the nonspecificity of the serologic assays, whereas others believe that it may be a cross-reaction with another agent. The massive waterborne outbreaks of acute hepatitis in New Delhi in the 1955 to 1956 period (7) were diagnosed as "classical"

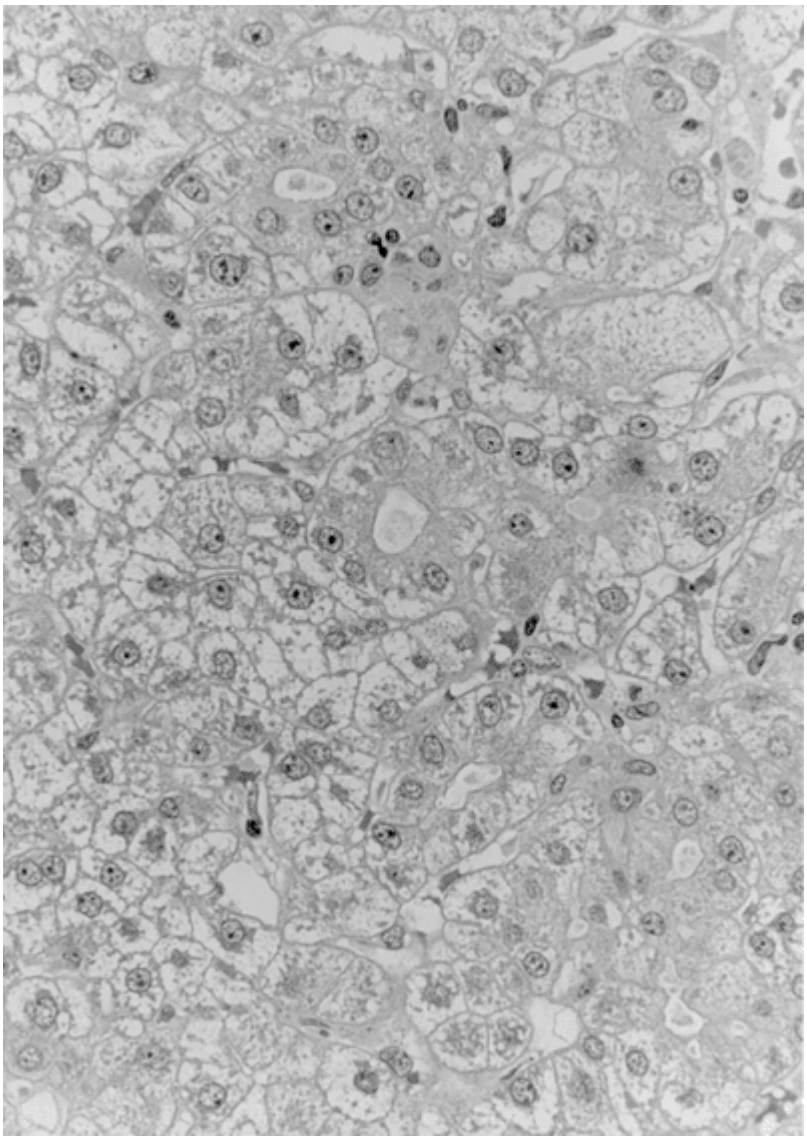
waterborne hepatitis A. However, serologic testing of the available specimens in 1980 ruled out acute hepatitis A or acute hepatitis B and allowed the recognition of the infection as non-A, non-B hepatitis (8). Since then at least 17 HEV outbreaks have occurred in India (9) and more than 50 in Asia, Africa, and the American continents (4). In 1975, sporadic cases from Costa Rica were reported in which the illnesses were neither hepatitis A nor hepatitis B (10). In 1986 and 1987, outbreaks of acute hepatitis occurred in two rural villages located 70 miles south of Mexico City; 223 cases were diagnosed as non-A, non-B hepatitis, and stool samples from some cases yielded viral particles 32 to 34 nm in size, similar to the enterically transmitted non-A, non-B hepatitis from Asia (11). Hepatitis E has been detected among US travelers to endemic regions (12). However, a unique HEV whose genome is significantly different from the Burmese or Mexican strains has been described in the United States in humans and swine (13).

In 2004, approximately 4,000 suspected cases of hepatitis E were reported by health clinics in Darfur, Sudan. Thousands of possible cases were also reported among refugees in Chad and Iraq (14). Hepatitis E is not an uncommon disease in many areas of the world, and it is recognized as a frequent cause of sporadic hepatitis in Asia.

Some species of animals (rodents, swine, monkeys, etc.) have been found to have detectable anti-HEV (15), raising the possibility of HEV being a zoonotic disease that can be acquired from animals; however, there is no confirmation of such transmission.

Pathogenesis

At least two human volunteers and studies in animal models have permitted the characterization of the pathogenesis of HEV. In the human volunteers, abnormal levels of aminotransferases were detected 4 to 5 weeks after ingestion of contaminated material and they remained abnormal for 1 to 3 months (2,16). Viral particles were excreted in stool approximately 4 weeks after ingestion of the inoculum, and the shedding lasted approximately 2 weeks when tested by immune electron microscopy. Using molecular biology techniques, shedding of viral particles has been observed close to 2 months after the ingestion. Immunoglobulin M (IgM) antibody to HEV parallels the rise of aminotransferase levels and declines in titer quickly, disappearing in a few weeks, although some patients may have detectable IgM anti-HEV for a few months. IgG anti-HEV level rises slowly and remains detectable for months, probably years after the infection.



▪ **Figure 28.1** Hematoxylin–eosin stain (×100) of a liver biopsy specimen from a 30-year-old Pakistani man with acute hepatitis E. Acinar transformation (gland-like) and cholestasis are observed along with necroinflammatory changes in the hepatic parenchyma.

Little is known about what happens once the virus is ingested; it is likely that the virus traverses through the small intestine and reaches the liver through the portal vein. The precise mechanism of hepatic uptake in humans is not known. Histopathologic features of HEV infection include necroinflammatory processes seen in all acute viral hepatitis and cholestatic hepatitis. HEV antigen was observed in the cytoplasm of infected hepatocytes as soon as 10 days after experimental intravenous inoculation and persisted for approximately 3

weeks (17). Interestingly, in some outbreaks, the cholestatic hepatitis has been described as a gland-like transformation of hepatocytes with bile stasis (Fig. 28.1).

The pathogenesis of HEV-associated hepatocyte injury is not completely defined.

Clinical Features

The clinical features of HEV are difficult to differentiate from those of other types of viral hepatitis. The incubation period ranges between 15 and 60 days, usually becoming symptomatic 40 days after exposure. Adults appear to be at a greater risk of developing symptoms than adolescents and children. Most patients experience malaise, lack of appetite, nausea, and vomiting. A third of infected patients experience fever or abdominal pain. Few patients experience diarrhea, arthralgias, or skin rash. Serum tests show abnormal levels of aminotransferases and hyperbilirubinemia. Most patients do well, and because there are no chronic sequelae, they recover fully. However, fulminant hepatitis is associated with HEV more often than with any other viral hepatitis (18), particularly among pregnant women. Case fatality rates among HEV-infected pregnant women have been reported between 15% and 25% (19,20). However, work with experimental pregnant animals failed to show differences in severity of HEV infection when compared to nonpregnant animals (21).

Diagnosis

Development of modern diagnostic tests was possible in part because of cloning of the virus (3) and the development of four recombinant viral antigens representing two distinct antigenic domains from two HEV strains (6). Before this advance, scientists relied on immune electron microscopy to detect viral particles in stool, with the consequent limitations of intensive labor and reduced sensitivity. Enzyme immunoassays are available to detect antibodies against HEV, and IgM or IgG classes of antibodies (6). These tests have an 80% to 100% probability of detecting the markers of acute infection (e.g., IgM anti-HEV). Data are limited to evaluate the longevity of IgG anti-HEV. Some studies have shown that IgG anti-HEV persists for at least 1 year after an acute infection (22); others have found that it may last longer. When 53 children from the outbreak in Mexico in the period 1986 to 1987 (11) were tested for anti-HEV 9 years later, 70% were found to have detectable IgG antibody (23).

Prevention and Treatment

No defined antiviral therapy exists, and treatment for acute HEV is mainly supportive. In cases of fulminant HEV, liver transplantation

should be considered.

Some investigators have evaluated the efficacy of pre- or postexposure to Igs as prophylaxis to prevent acute HEV hepatitis. Unfortunately, even when using Igs from endemic regions (e.g., India) there was no clinical benefit observed (24). Prevention against HEV infection is associated with hygienic measures and clean water. No vaccine for human use is commercially available yet, but several recombinant HEV proteins have been evaluated as vaccine candidates. Table 28.2 shows some of these vaccines (25).

Table 28.2. Status of Hepatitis E Vaccines

Type	Pharmaceutical company or research group	Clinical stage
56-kDa ORF2 protein VLPs (baculovirus)	GlaxoSmithKline/United States Army/United States National Institutes of Health	Phase II, III
DNA vaccine	United States Navy	Preclinical
Live swine virus vaccine	United States National Institutes of Health	Preclinical

ORF, open reading frame; VLP, virus-like particles; DNA, deoxyribonucleic acid.
Modified from World Health Organization. *New vaccines against infectious diseases: research and development status*, April 2005; http://www.who.int/vaccine .

GlaxoSmithKline (GSK) Biologicals has been working on the development of a hepatitis E vaccine to protect adolescents and adults. The vaccine is a 56-kDa recombinant product, expressed in insect cells from a baculovirus vector, initially developed at the National Institutes of Health (NIH), United States, and subjected to successful phase I studies (26). Recently, a blinded placebo-controlled study has been conducted in Nepal, involving 2,000 adults from the Royal Nepalese Army. The study is a collaboration between GSK, The United States Army, the Royal Nepalese Army, and the US NIH. The vaccine was administered as three injectable doses at 0, 1, and 6 months, and it was shown to be safe and immunogenic. The vaccine efficacy was

calculated to be 97% among vaccine recipients because only three subjects among the 898 who were fully immunized developed acute HEV, while 66 of the 896 placebo recipients had acute HEV (Robert H. Purcell, *personal communications* 2006). The prospect of controlling hepatitis E is likely a reality in the near future.

Annotated References

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Purcell RH, Nguyen H, Shapiro M, et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* 2003;21:2607–2615.

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Experimental animal infectious bile was used to construct HEV recombinant libraries. The clone ET1.1 was characterized and found to represent a portion of the HEV genome.

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Chapter 29

Hepatitis B and D

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Key Concepts

- There are approximately 400 million hepatitis B carriers worldwide and 1.25 million carriers in the United States. The prevalence of hepatitis B virus (HBV) infection is related to the predominant mode of transmission and the age at infection.
- Acute HBV infection may manifest as subclinical hepatitis, icteric hepatitis, or fulminant hepatitis. Chronic HBV infection may manifest as inactive carrier state, chronic hepatitis, cirrhosis, or hepatocellular carcinoma (HCC).
- Many lines of evidence support an etiologic association between chronic HBV infection and HCC.
- The aims of antiviral treatment of chronic hepatitis B are to suppress HBV replication, induce remission in liver disease, and prevent the development of cirrhosis and (HCC).
- Approved treatments of chronic hepatitis B include interferon-α (standard and pegylated), lamivudine, adefovir, and entecavir.
- Hepatitis D virus is dependent on HBV. Hepatitis D occurs as coinfection with HBV or superinfection in persons with chronic HBV infection.

Epidemiology

Hepatitis B infection is a global public health problem. It is estimated there are approximately 400 million hepatitis B virus (HBV) carriers in the world, of whom over 500,000 die annually from hepatitis B–associated liver disease (1). In the United States, an estimated 1.25 million individuals are chronically infected with HBV (2,3). Hepatitis B carrier rate varies from 0.1% to 20% in different areas of the world (Table 29.1). The wide range in carrier rate is related to differences in the predominant mode of transmission and the age at infection. Understanding the epidemiology of hepatitis B is important in the implementation of vaccination programs for the prevention of HBV infection.

Prevalence

The prevalence of HBV infection varies in different geographical areas (Table 29.1) (4). In low prevalence areas such as the United States, western Europe, Australia and New Zealand, the hepatitis B surface antigen (HBsAg) carrier rate is approximately 0.1% to 2%. In intermediate prevalence areas like the Mediterranean countries, Japan, India and Singapore, the carrier rate is approximately 3% to 5%. In high prevalence areas such as Southeast Asia and sub-Saharan Africa, the carrier rate is 10% to 20%. The prevalence of current and past HBV infection is estimated to be 5% in the United States and close to 100% among adults in some parts of Southeast Asia and Africa. In general, there is an increasing prevalence of HBV infection with age. Within the United States, the prevalence of HBV infection is higher among African Americans, Hispanics and Asians than in the white population (3). Several communities have been reported to have higher carrier rates than their neighboring regions, namely, Alaskan Eskimos, Asian-Pacific Islanders and Australian Aborigines.

In most high prevalence areas such as Hong Kong and China, perinatal transmission is the major mode

of spread accounting for 40% to 50% of chronic HBV infection (5,6). However, horizontal spread during the first 2 years of life is the major mode of transmission in other endemic areas including Africa and the Middle East (7,8). The exact reason for the preponderance of perinatal transmission among Orientals is not clear but is at least in part related to the high prevalence of hepatitis B e antigen (HBeAg) among Asian carriers of reproductive age—40% to 50% versus 10% to 20% among African carriers of the same age-group (6,9). In intermediate prevalence areas, transmission occurs among all age-groups but early childhood infection accounts for most cases of chronic infection. In low prevalence areas, most infections are acquired in early adult life through unprotected sexual intercourse or intravenous drug abuse. The age at infection has a significant impact on the clinical outcome because chronic infection occurs in

approximately 90% of infants infected at birth, in 25% to 50% of children infected between the age of 1 and 5 years, and in less than 5% of those infected during adult life (5,10,11,12).

Table 29.1. Patterns of Hepatitis B Virus Infection

Prevalence	High	Intermediate	Low
Carrier rate	8%–20%	3%–7%	0.1%–2%
Geographical distribution	Southeast Asia China Pacific Islands Sub-Saharan Africa Alaska (Eskimos)	Mediterranean basin Eastern Europe Central Asia Japan Latin and South America Middle East	United States and Canada Western Europe Australia New Zealand
Predominant age at infection	Perinatal and early childhood	Early childhood	Adult
Predominant mode of	Maternal–infant	Percutaneous	Sexual
transmission	Percutaneous	Sexual	Percutaneous

Mode of Transmission

Transfusion

In the 1960s, the risk of hepatitis B infection from transfusions of commercial blood was as high as 50% and HBsAg was detected in up to 60% of patients with post-transfusion hepatitis. The exclusion of paid donors and the application of hepatitis B serologic screening in the 1970s dramatically reduced the incidence of post-transfusion HBV infection (13). Currently, approximately 80 cases of transfusion-associated HBV infection are reported in the United States each year (14). In the United States, both HBsAg and hepatitis B core antibody (anti-HBc) are used for blood donor screening. Anti-HBc was initially used as a surrogate marker for non–A non–B hepatitis virus. Anti-HBc has been retained after the implementation of hepatitis C testing to detect donors who are in the window phase during recovery from acute hepatitis B or who have low level chronic HBV infection. The practical value of anti-HBc screening is not clear because of the possibility of false-positive test result, the low incidence of transfusion-associated HBV infection with HBsAg screening only in low prevalence areas, and the need to exclude as many as 22% of the donor population in high prevalence areas (15,16,17,18). Currently, the risk of transfusion related hepatitis B from blood donors who test negative for HBsAg and anti-HBc is estimated to be 1 in 63,000 (range 1:30,000 to 1:250,000 episodes per unit transfused) (19).

Recently there have been debates on serology versus nucleic acid testing (NAT) of blood donors. A recent review concluded that NAT for HBV will probably detect only a few more donor units that may be associated with risk of transmitting HBV infection compared to serologic screening for HBsAg and anti-HBc (20). With the current estimated 13 million donations per year in the United States and 1.8 transfused components per donation, introduction of NAT would be expected to prevent 30 to 35 HBV-containing transfusions per year. Because of the low rates of viral persistence and clinical disease following HBV transmission in the setting of seronegative blood transfusion, the clinical impact and cost-effectiveness of NAT is expected to be low. NAT of whole blood was estimated to avert 9 to 37 HBV infections at an additional cost of US $39 to 130 million per year (21).

Percutaneous transmission

Percutaneous inoculation of blood or body fluid plays a major role in the transmission of hepatitis B infection. Needle sharing by intravenous drug users is an important route of transmission of hepatitis B. Reuse of contaminated needles for tattoos, acupuncture and ear piercing also provide opportunities for percutaneous transmission.

Sexual transmission

In the United States and many developed countries, sexual transmission is the most important mode of spread

of HBV. The Centers for Disease Control and Prevention reported that sexual transmission accounts for almost 50% of acute HBV infection among individuals in whom data on risk factors were available (22). A high prevalence of chronic HBV infection has been reported in men who have sex with men as well as in heterosexuals with multiple sex partners. The annual incidence of new HBV infections among homosexual men decreased significantly in the 1980s as a result of education on safe sex practice to prevent human immunodeficiency virus (HIV) infection (23). However, recent reports in the United States suggest that both heterosexual transmission and transmission among homosexual men are on the rise (22). The risk of sexual transmission of HBV infection is proportional to the number of lifetime sex partners, low education level, paid sex, and history of sexually transmitted diseases.

Perinatal transmission

The rate of neonatal HBV infection from an infected mother is less than 10% in Western countries. Nonetheless, an estimated 20,000 infants are born to HBsAg carrier women in the United States annually (24). In areas with high endemicity such as China, perinatal infection is the most common mode of transmission. The risk of maternal–infant transmission is related to the HBV replicative status of the mother. The risk is 85% to 90% for infants born to HBeAg-positive mothers and 30% for infants born to HBeAg-negative mothers (25). More recent studies demonstrated that maternal serum HBV DNA levels correlate better with the risk of transmission (26). Maternal–infant transmission takes place at the time of delivery by maternal–fetal transfusion or exposure to maternal blood during passage through the birth canal and postnatally through intimate mother–baby contact. Intrauterine transmission is uncommon as HBsAg is detected in infants much later. In addition, passive–active immunization at birth has been demonstrated to have an efficacy rate of more than 90% in the prevention of HBV infection (27). Cesarean section has not been shown to eliminate the risk of perinatally acquired HBV infection (28) and should not be routinely recommended for carrier mothers. Although HBsAg can be detected in breast milk, there is no evidence that HBV infection can be transmitted by breast-feeding (29); infants born to carrier mothers may be breast-fed if they have been vaccinated. The risk of transmission during amniocentesis is also low (30). Universal vaccination of all newborns and additional administration of hepatitis B immune globulin (HBIG) to those who are born to carrier mothers were initiated in many Southeast Asian countries in the 1980s. These programs have led to significant reduction in HBsAg carrier rate as well as decrease in the incidence of hepatocellular carcinoma (HCC) among children (31).

Health care environment

HBV is the most commonly transmitted blood-borne virus in the health care setting (32). Transmission generally occurs from patient to patient or from patient to health care personnel via contaminated instruments or accidental needle stick injury. The risk of acquiring HBV infection after needle stick injury is related to the HBeAg status of the source patient. There have been several outbreaks of hepatitis B infection in the health care environment. One report involved transmission from a patient with diabetes to another through the contaminated platform of a spring-loaded lancet device for finger sticks (33). Outbreaks of HBV infection were also reported in several hemodialysis units as a result of failure to identify and isolate patients who were infected and to vaccinate those who were susceptible (34). Transmission of HBV infection from health care workers to patients is rare. One outbreak was traced to a cardiothoracic surgeon despite no identified flaws in precautions on infection control during operations (35). Transmission was thought to be related to tears in the gloves and minor cuts on the surgeon's fingers during prolonged suturing. Nosocomial transmission can be prevented by screening of blood and blood products, use of disposable needles and equipment, proper sterilization of surgical instruments, enforcement of infection control measures, and vaccination of health care workers.

In many developed countries, guidelines have been established to define the parameters within which health care workers with hepatitis B can operate. In the United States, health care workers who are HBeAg positive are restricted from performing invasive procedures (36,37). The Centers for Disease Control and Prevention recommends that health care workers with HBV infection should not perform exposure prone procedures unless they have sought counsel from an expert review panel and have been advised on the circumstances under which they may perform such procedures. The difference in the scope of permissible work between HBeAg-positive and HBeAg-negative carriers is related to the traditional concept that HBeAg is a reliable marker of infectivity. However, a recent report found that transmission of HBV infection occurred from four HBeAg-negative surgeons. These surgeons had detectable HBV DNA in serum and were infected with precore stop codon variants (38). This and other similar incidents have led to the proposal that serum HBV DNA levels be used to categorize the infectivity of health care workers but it is also known that serum HBV DNA levels can fluctuate and may be intermittently undetectable in patients with chronic HBV infection (37,39,40). As vertical transmission is rarely documented with maternal HBV DNA levels below 10^7 copies/mL, it is thought that transmission of HBV

via needle-stick injury is also unlikely to occur at HBV DNA levels below 10^7 copies/mL (39). It has been proposed that health care workers with higher HBV DNA levels receive antiviral therapy to enable them to return to work without risking nosocomial infection.

Hemodialysis patients

Patients with renal failure on hemodialysis may be infected through blood transfusions, contamination of dialysis machines or equipment, as well as interpersonal horizontal transmission in the dialysis units. Improved infection control and the availability of vaccines have reduced the incidence of HBV infection among hemodialysis patients from 3% in 1980 to 0.1% in 1993 in the United States and has remained stable in the past decade (34,41). However, dialysis patients have impaired antibody response to vaccines. Therefore, vigilance is still needed to prevent outbreaks.

In a recent survey of all US chronic hemodialysis centers (41) the percent of patients vaccinated against HBV infection increased from 47% to 56% and the percent of staff vaccinated increased from 87% to 90% between 1997 and 2002. Although the overall incidence of HBV infection did not correlate with the infection control practices, it was noted that the incidence of HBV infection in 2002 was higher among patients in centers where injectable medications were prepared on a medication cart compared to a dedicated medication room.

A possible contributing factor for continued transmission of HBV infection in adult hemodialysis units appears to be the presence of occult HBV infection (serum HBsAg negative but HBV DNA positive). In a recent study of 241 adult hemodialysis patients in a North American urban center (42), only two patients (0.8%) were HBsAg-positive but nine (4%) HBsAg-negative patients were HBV DNA positive.

Transplantation

Currently, organ donors are routinely screened for HBsAg. Transmission of HBV infection has been reported after transplantation of extrahepatic organs such as kidneys from HBsAg-positive donors. This may be related to residual blood in the vascular pedicles due to inadequate flushing or the presence of infectious virions in the kidneys. Transmission of HBV infection has also been reported after transplantation of avascular tissues such as cornea (43).

The role of anti-HBc testing in organ donor screening is uncertain because of the possibility of false-positive results, the potential loss of up to 5% of donors even in low endemic areas (44), and the uncertainty about the infectivity of organs from donors who have isolated anti-HBc (45). The incidence of HBV infection from donors with isolated anti-HBc is very low (0% to 2%) in heart and kidney recipients but varies from 0% to 78% in liver recipients (44,46,47,48). A recent study found that the estimated probability of undetected hepatitis B viremia is higher among tissue donor**s** compared to first-time blood donors and the addition of NAT to the screening of tissue donor**s** is expected to reduce the risk of HBV infection (49).

Others

In endemic areas, horizontal transmission among children may result from close bodily contact leading to transfer of virus across minor skin breaks and mucous membranes. Blood-feeding insects like mosquitoes have been demonstrated to serve as vectors for HBV transmission in animal models but firm evidence for this mode of transmission in humans is lacking. Various body secretions have been reported to test positive for HBsAg but only semen and saliva have been consistently shown to harbor infectious virions (50,51). Although HBV DNA has been detected in the saliva of some hepatitis B carriers, there is no convincing evidence that hepatitis B can be transmitted orally (52,53). As HBV survives for a long time outside the human body, transmission via contaminated environmental surfaces and daily articles such as toothbrushes, razors, eating utensils or even toys may also be possible.

High-Risk Groups

Health care workers have a higher hepatitis B carrier rate than the general population. The prevalence is particularly high among surgeons, pathologists, and physicians working in hemodialysis and oncology units. Apparent skin breaks, minor cuts and accidental needle stick injuries serve as portals of entry. Other health care workers having increased risk of HBV infection include dentists and laboratory personnel who have contact with serum. Institutionalized mentally handicapped persons as well as their attendants and family members also have a high rate of hepatitis B infection. Despite screening of blood products, patients requiring frequent transfusion of blood or blood products—those with thalassemia and hemophilia—have an increased risk of contracting hepatitis B infection. Other high-risk groups include intravenous drug users particularly those who share syringes, men who have sex with men and promiscuous heterosexuals, immigrants from HBV endemic countries, and spouses, sexual partners, and household members of HBV carriers.

Changing Epidemiology

The worldwide incidence of HBV infection is decreasing (1,22). Mass vaccination for newborns and catch-up vaccination for children and adolescents play a major role in reducing HBV infection among infants and

children. Increased public awareness of hepatitis, educational campaigns to prevent HIV infection leading to modification of high risk sexual behavior, and reduction of syringe sharing among intravenous drug

users have contributed to the decrease in HBV infection among adults.

In the United States, the incidence of acute hepatitis B has significantly declined over the past decade. According to the Centers for Disease Control and Prevention, the incidence of acute hepatitis B during the years 1990 to 2002 has declined from 8.5 per 100,000 population to 2.8 per 100,000 population (22); the most significant decline was seen among ages 0 to 19 years (rate of 3.0 to 0.3). The decline was more marked in women compared to men. However, incidence has remained the same if not increased among certain adult groups: Those with multiple sexual partners, men who have sex with men, and injection drug users. Sexual transmission among susceptible individuals remains a significant risk factor for hepatitis B transmission in the United States. This is in part related to lack of resources and infrastructure for vaccination of adults as well as missed opportunities. In a recent study of 833 men who have sex with men, aged 15 to 29 years, 44% were susceptible to HBV infection; most of these men were found to be either unaware of protective vaccines, had never been offered vaccination, or perceived themselves at low risk (54).

Another important aspect of HBV epidemiology is that in many developed countries, immigrants from countries that are endemic for HBV infection now constitute an increasing proportion of those with chronic HBV infection (55). In addition, some studies also showed that these immigrants have a higher incidence of acute HBV infection (56). These and other studies (57) suggest that screening and immunization of susceptible adults along with immunization of children (especially if they were born in countries where universal vaccination is not in place) whose parents immigrated from HBV endemic countries may be of great importance in controlling HBV infection in developed countries.

Vaccination

Indications

Vaccination against hepatitis B remains the mainstay of prevention. Universal vaccination of all newborn or at least newborn of all HBV-infected mothers is currently practiced in most countries throughout the world. The World Health Organization (WHO) has recommended that combination of hepatitis B and childhood vaccines be used where possible, to reduce the logistic costs of vaccine delivery especially in areas where it is most needed. However, due to decreased immunogenic potential of other vaccines especially during the initial 6 weeks after birth, it is currently recommended that only monovalent vaccines should be administered to the newborn. In some developed countries, where universal vaccination of all newborn is not in place, vaccination of adolescents to prevent sexual transmission is implemented. Vaccination of adults is recommended for high risk groups including health care workers, men who have sex with men, persons with multiple sex partners, injection drug users, sex partners, and household members of HBV carriers, public safety workers, institutionalized patients, and patients on chronic hemodialysis (Table 29.2) (58).

Table 29.2. Indications for Hepatitis B Vaccine

1. All newborns[a]
2. All children and adolescents not vaccinated at birth
3. High-risk adults:
    a. Health care workers
    b. Men who have sex with men
    c. Persons with multiple sexual partners
    d. Injection drug users
    e. Patients on hemodialysis
    f. Institutionalized patients
    g. Health care workers and public safety workers
    h. Spouse, sexual partners and household members of HBV carriers

[a]For infants born to carrier mothers, hepatitis B immune globulin (HBIG) is also administered at birth.

Administration Schedule

There are two types of hepatitis B vaccines, plasma-derived and recombinant, the latter is currently used in most countries. Recombinant HBV vaccines consist of HBV small S protein (HBsAg) produced by yeast or mammalian cells. Hepatitis B vaccine is usually administered intramuscularly in three doses at 0, 1 and 6 months, the dose being 10 to 20 μg in adults and 5 to 10 μg in children (Table 29.3). For adults, the injections are given in the deltoid muscle, whereas in newborns and young children the recommended site is the anterolateral thigh. In patients with hemophilia,

it is recommended to administer the vaccine subcutaneously.

Table 29.3. Hepatitis B Vaccines and Dosage Recommendations

Vaccine brand	Age-group (y)	Dose (μg)	Volume (mL)	Number of doses
Engerix-B	0–19	10	0.5	3
	≥20	20	1.0	3
Recombivax HB	0–19	5	0.5	3
	≥20	10	1.0	3
(Optional two doses)	11–15	10	1.0	2
For hemodialysis patients, recommended dose is 40 μg with each dose (Engerix-B 40 μg/2.0 mL and Recombivax HB dialysis formulation 40 μg/1.0 mL).				

For infants born to HBsAg-negative mothers and unvaccinated children/adolescents up to 19 years of age, 3 doses (0, 1 and 6 months) of vaccine at half strength should be administered. For adults 20 years and older, the same regimen is implemented using full dose (10 μg of Recombivax HB and 20 μg of Engerix-B). An alternative two-dose schedule had been approved for adolescents.

For newborns of HBsAg carrier mothers, HBIG 0.5 mL and the first dose of vaccine should be administered at birth, using different sites. Combination of HBIG and hepatitis B vaccine has been shown to be 95% efficacious in preventing perinatal transmission of HBV infection (27,59,60).

For patients on hemodialysis or immunocompromised patients, higher doses of vaccine are needed: 40 μg of Recombivax HB or Engerix-B. Anti-HBs titer should be monitored annually, and booster doses administered when hepatitis B surface antibody (anti-HBs) titer falls below 10 IU/L.

Follow-up testing for protective antibodies is recommended for individuals who continue to be at risk including infants born to HBsAg-positive mothers, health care workers, hemodialysis patients, and sexual partners of HBsAg carriers (58).

Some vaccines have also incorporated pre-S1 (large S) and/or pre-S2 (middle S) proteins to increase the immunogenicity but these vaccines are not available in most countries.

Efficacy

A protective response defined as an anti-HBs titer more than 10 IU/L is achieved in approximately 95% of vaccine recipients. Several studies have shown that vaccination is effective in inducing protective immunity and in preventing HBV infection even in men who have sex with men, (61,62,63) and newborns of carrier mothers (27,59,64). In countries where the prevalence of HBeAg among carrier mothers is low, it has been shown that the vaccine alone has similar efficacy in preventing HBV infection as a combination of vaccine and HBIG in preventing perinatal infection (64). Although this approach can be cost saving, it may not be adequate in countries where the prevalence of HBeAg among carrier mothers is high or in countries where a high percent of HBeAg-negative mothers have high serum HBV DNA levels.

Factors Associated with Nonresponse and Management of Vaccine Nonresponders

Approximately 2.5% to 10% of vaccine recipients fail to respond with adequate anti-HBs titers after one course of HBV vaccine. The reasons for nonresponse are several and include older age, obesity, chronic medical illnesses such as renal failure, diabetes, cirrhosis, immunosuppression such as patients with HIV infection or organ transplantation, and technical problems such as intragluteal injection and inadvertent freezing of the vaccines.

Nonresponse to HBV vaccine has been reported to be associated with impaired lymphocyte activation as well as genetic factors including certain human leukocyte antigen (HLA) class II genes such as HLA-DRB1*0301 and cytokine gene polymorphisms (65,66,67).

For individuals who failed to respond after a full course of vaccination the recommendation is to repeat another course of vaccine. If a person still remains a nonresponder, further vaccination is usually not effective but most of these individuals can mount an adequate immune response upon infection because

exposure to HBV stimulates both T and B cell responses to HBsAg as well as hepatitis B core antigen (HBcAg). Nonresponders to two courses of vaccine should be tested for HBsAg as some may be undiagnosed carriers.

Durability of Vaccine Response and Need for Boosters

Several studies showed that 30% to 66% of individuals had protective levels of anti-HBs (≥10 mIU/mL) for even 15 years or more after receiving plasma derived HBV vaccines and 90% had anamnestic response after booster vaccination (68,69,70,71). Breakthrough infections appear to occur mostly among those who did not have an initial response to vaccination (70). Two recent studies found that persistence of anti-HBs response up to 18 years after administering plasma-derived and recombinant vaccines was comparable (68,72).

Although anti-HBs titers decline with time, the incidence of HBV infection among individuals who were vaccinated at birth is low and there is no consensus on the need for booster vaccination. The European Consensus Group on hepatitis B Immunity in 2000 recommended that booster doses be considered in those who are immunocompromised or at a high risk of exposure (73). A recent report of the Steering Committee for the prevention and control of infectious diseases in Asia (74) recommended booster vaccination approximately 10 to 15 years after primary vaccination especially among children vaccinated as infants; when monitoring of antibody levels is not feasible; in all immunocompromised patients with anti-HBs levels below 10 mIU/L; and for health care workers in endemic countries. By contrast, a Viral Hepatitis Prevention Board that convened in 2004 concluded that existing data do not support the need for booster doses in universal HBV immunization programs, but the risk of

infection through sexual or occupational exposure later in life on those vaccinated as neonates is unknown (75).

Impact of Hepatitis B Virus Vaccination

HBV vaccination has been shown to reduce the incidence of acute HBV infection and HCC, and the prevalence of chronic HBV infection (31,57,76,77). HBV vaccine is the first vaccine that has been shown to prevent cancer (HCC) in humans. After the implementation of a nationwide vaccination program for newborn and children in 1984, the carrier rate among children decreased from 10% in 1984 to less than 1% in 1999 (31) while the incidence of HCC declined from 0.79 cases per 100,000 between the years 1981 and 1986 to 0.36 cases between the years 1990 and 1994 (77). In the United States, universal vaccination of all newborns was implemented in 1991 and it was expanded to include vaccination of all adolescents aged 11 to 12 years in 1995, and children aged less than 18 years, who had not been vaccinated previously, in 1999. This has resulted in a significant 89% reduction of acute hepatitis B in children and adolescents during 1990 to 2002 (22).

Safety of Hepatitis B Vaccination

The safety of hepatitis B vaccination has been well established. The most common adverse reaction is soreness over the injection site. Other adverse reactions include low-grade fever, malaise, headache, arthralgia and myalgia. Hepatitis B vaccines have no teratogenic effects and can be administered during pregnancy (78,79).

There has been concern about the possibility of hepatitis B vaccine leading to the development of demyelinating central nervous system diseases including multiple sclerosis (80,81) and also Guillain-Barre syndrome (82). It has been speculated that these "adverse reactions" could be related to "molecular mimicry." However, many studies have failed to show a statistically significant temporal or causal association between HBV vaccine and these neurologic or immunologic conditions (83,84,85,86,87). Because of concerns about mercury exposures, current preparations of HBV vaccines do not contain thimerosal as a preservative.

Based upon current evidence and the proven benefit of hepatitis B vaccine, the WHO has recommended that all countries continue their hepatitis B vaccine programs (88).

Special Settings

Isolated antihepatitis B core individuals

The presence of an isolated anti-HBc does not always denote prior exposure to HBV infection. HBV vaccination has been recommended to differentiate those who had prior exposure from those with false-positive anti-HBc test results (15). With improved specificity of current anti-HBc assays, most individuals with isolated anti-HBc have genuinely positive test results and do not need to be vaccinated but there is no harm if vaccine is administered.

Patients on chronic hemodialysis

Response to HBV vaccine is impaired in patients with renal failure. A recent report from the Cochrane group found that there was no difference in response between plasma derived and recombinant HBV

vaccines (89). Response to HBV vaccine was similar in hemodialysis versus peritoneal dialysis patients (90).

Patients with chronic liver disease

Hepatitis B vaccination along with vaccination against hepatitis A is currently recommended for all patients with underlying chronic liver disease. Acute hepatitis B superimposed on chronic hepatitis C has been reported to be associated with increased risk of liver failure (91). Immune response to HBV vaccines among patients with chronic liver disease varies from 70% to 90% (91). In general, response rates are similar to healthy subjects with no liver disease except in patients with cirrhosis but response rates are substantially lower (<50%) in patients with decompensated cirrhosis awaiting liver transplantation (92,93). Therefore, it is recommended that HBV vaccination should be administered early, before a patient develops cirrhosis.

Patients with human immunodeficiency virus infection

Several reports have suggested that patients with HIV infection have a blunted response to HBV vaccine compared to HIV-negative individuals. A recent large randomized, double-blind study of two doses of recombinant HBV vaccine (standard dose of 20 µg and double dose of 40 µg) (94) showed that a response was seen in 34% and 47% in the standard and double dose groups, respectively. Response rates were higher in those with high CD4 count and low HIV RNA level.

Novel methods of vaccine administration

In an effort to reduce the number of injections and increase compliance, several combination vaccines have been developed. They include combinations of hepatitis A and B vaccine for adults and children, and a hexavalent combined vaccine against diphtheria, pertussis, tetanus, polio, *Haemophilus*, and hepatitis B for children. These combined vaccines have been shown to be well tolerated and safe. They have comparable rates of development of protective antibody levels compared to monovalent vaccines (95,96).

Various strategies have been examined to improve immunogenicity of HBV vaccines. One approach is to use more potent adjuvants (97,98). Another approach is to activate mucosal T cells through nasal vaccination (99). Other approaches include intradermal administration, coadministration with interleukin-2, and incorporation of pre-S1 and/or pre-S2 antigens (100,101,102,103).

Diagnosis

The diagnosis of hepatitis B was revolutionized by the discovery of Australia antigen, now called *HBsAg*, by Blumberg in 1965 (104). During the ensuing decade, serologic assays for HBsAg and anti-HBs with increasing sensitivity and specificity were developed. In the 1970s, additional HBV antigens and antibodies were identified and serologic assays for their detection established. Advances in molecular biology techniques in the 1980s led to the development of hybridization assays for direct determination of virus replication and polymerase chain reaction (PCR) assays that permitted the detection of as little as ten molecules of HBV DNA per mL of serum. Diagnosis of HBV infection can also be made by the detection of HBsAg or HBcAg in liver tissues by immunohistochemical staining and of HBV DNA by Southern hybridization, in situ hybridization or PCR.

Serologic Diagnosis

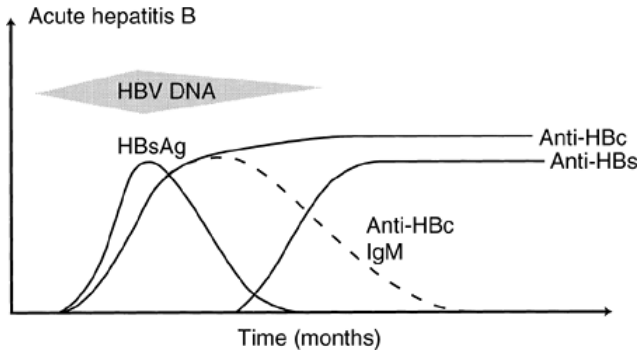
Serological markers during HBV infection are shown in Figures 29.1 and 29.2 and Table 29.4.

Hepatitis B surface antigen and hepatitis B surface antibody

HBsAg is the serologic hallmark of HBV infection. It can be detected by radioimmunoassays (RIAs) or enzyme immunoassays (EIAs). HBsAg appears in serum 1 to 10 weeks after an acute exposure to HBV, approximately 2 to 6 weeks before the onset of hepatitis symptoms or elevation of alanine aminotransferase (ALT) (105). In patients who subsequently recover, HBsAg usually becomes undetectable after 4 to 6 months. Persistence of HBsAg for more than 6 months implies chronic infection. The disappearance of HBsAg is followed by the appearance of anti-HBs. Although anti-HBs is produced early in the course of acute infection in individuals who subsequently recover, they are frequently not detectable until after a window period of several weeks to months when neither HBsAg nor anti-HBs can be detected (105) (Fig. 29.1). The appearance of anti-HBs marks the recovery from hepatitis B. In most patients, anti-HBs persist for life, therefore conferring long-term immunity. Anti-HBs is the only protective antibody induced by most of the currently available vaccines, which consist of recombinant HBsAg only.

Table 29.4. Interpretation of Hepatitis B Virus (HBV) Serologic Markers

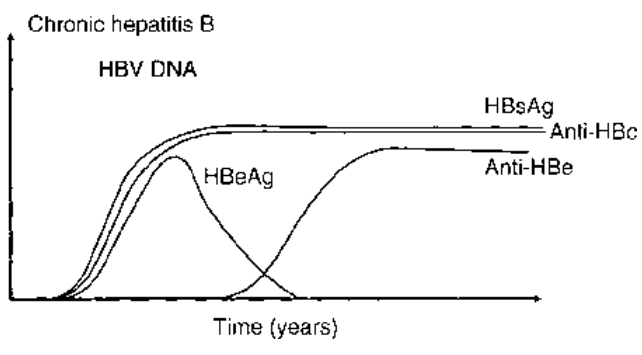
Hepatitis B surface antigen	HBV infection: acute or chronic
Hepatitis B e antigen	High levels of HBV replication and infectivity
Anti-HBe	Low levels of HBV replication and infectivity
Anti-HBc (IgM)	Recent HBV infection
Anti-HBc (IgG)	Recovered or chronic HBV infection
Anti-HBs	Immunity to HBV infection
Anti-HBc (IgG) + anti-HBs	Past HBV infection
Anti-HBc (IgG) + HBsAg	Chronic HBV infection



▪ **Figure 29.1** Serologic markers during acute hepatitis B virus infection. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; anti-HBs, hepatitis B surface antibody; IgM, immunoglobulin M.

HBV can be classified into eight genotypes (106,107) and four major serotypes (108). All HBV serotypes share one common antigenic determinant, "a," which is a conformational epitope located in the HBsAg. There are two additional pairs of mutually exclusive subtypic determinants "d" or "y" and "w" or "r" constituting the four major serotypes—adr, ayr, adw, and ayw. Antibodies to the "a" determinant confer protection to all HBV serotypes (109). At least 50% of the anti-HBs

that develops after recovery from acute hepatitis B or immunization with hepatitis B vaccines are directed against the "a" determinant, therefore providing cross-protection against other serotypes of HBV.



▪ **Figure 29.2** Serologic markers during chronic hepatitis B virus infection. HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBc, hepatitis B core antibody; anti-HBe, hepatitis B e antibody.

Coexistence of HBsAg and anti-HBs has been reported in approximately 24% of HBsAg-positive individuals (110). In most instances, the antibodies are directed against one of the subtypic determinants and not the common "a" determinant and are unable to neutralize the circulating virions (111). These individuals should therefore be regarded as carriers.

Pre-S1 and pre-S2 antigens have been detected in patients infected with HBV. In general, the presence of these antigens correlates with the detection of HBV DNA and virus replication (112). During recovery from acute hepatitis B, antibodies to pre-S1 and pre-S2 antigens appear early (113), prior to the detection of anti-HBs.

Hepatitis B core antigen and antihepatitis B core antibody

HBcAg is an intracellular antigen that is expressed in infected hepatocytes. It is not detectable in serum. Its antibody—anti-HBc—however, can be detected throughout the course of HBV infection. During acute HBV infection, anti-HBc is predominantly immunoglobulin (Ig) M class. IgM anti-HBc is the first antibody to be detected (Fig. 29.1). It usually appears within 1 month after the appearance of HBsAg, approximately 1 to 2 weeks before ALT begins to rise (105). It is the sole marker of HBV infection during the window period, that is, the time gap between the disappearance of HBsAg and the appearance of anti-HBs (114). During convalescence, the titer of IgM anti-HBc declines while the titer of IgG anti-HBc increases. Therefore, the detection of IgM anti-HBc is usually taken as an indication of acute HBV infection. However, in 20% of patients, IgM anti-HBc may remain detectable up to 2 years after the acute infection (115). In addition, low-titer IgM anti-HBc persists in most patients with chronic HBV infection. Therefore, the reliability of IgM anti-HBc in the differentiation of acute from chronic HBV infection depends on the cutoff level in the assay. Even in assays with high cutoff values, IgM anti-HBc can be detected in patients with chronic HBV infection during exacerbations (116). This may lead to misdiagnosis of acute hepatitis B in patients who are not previously known to have chronic HBV infection and overestimation of the rate of progression to chronicity. Recent studies in endemic areas demonstrated that many patients presenting with acute hepatitis who test positive for HBsAg have exacerbations of chronic hepatitis B and not acute hepatitis B, and that less than 1% of immunocompetent adult patients with genuine acute hepatitis B progress to chronic infection (12,117).

IgM anti-HBc titers have been reported to correlate with ALT and serum HBV DNA levels in patients with chronic hepatitis B especially during exacerbations (118). It has also been suggested that serial IgM anti-HBc titers may be useful in monitoring response to interferon (IFN) therapy (119).

IgG anti-HBc persists along with anti-HBs in patients who recover from acute hepatitis B and in association with HBsAg in those who progress to chronic HBV infection.

Isolated presence of anti-HBc in the absence of HBsAg and anti-HBs has been reported in 0.4% to 1.7% among blood donors in low prevalence areas (120,121) and in 10% to 20% of the population in endemic countries (15,122). Isolated detection of anti-HBc may occur during the window period of acute hepatitis B when the anti-HBc is predominantly IgM class, many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels, or after many years of chronic HBV infection when HBsAg titer has decreased below the level for detection. The clinical significance of isolated anti-HBc is complex. Although HBV DNA has been detected in the serum of individuals with isolated anti-HBc when tested by PCR assays, the frequency of detection varies from 0% to 20% (18,123,124). Transmission of HBV infection has been reported from blood and organ donors with isolated anti-HBc but the incidence ranged from 0.4% to 78% (18,47,48,125,126), the risk being highest when livers from anti-HBc-positive donors are transplanted into seronegative recipients. Several studies found that 50% to 70% of asymptomatic individuals with isolated anti-HBc have false-positive test results (15,16), the false-positive rate has decreased with improved anti-HBc assays. The evaluation of individuals with isolated anti-HBc should include repeat testing for anti-HBc, HBsAg, anti-HBs, and hepatitis B e antibody (anti-HBe). Individuals with evidence of chronic liver disease should be tested for HBV DNA to exclude low-level chronic HBV infection.

Hepatitis B e antigen and hepatitis B e antibody

HBeAg is a secretory protein that is processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. Its presence is usually associated with the detection of HBV DNA polymerase (127) and HBV DNA (128) in serum. Epidemiologic studies reported significantly higher rates of transmission of HBV infection from HBeAg-positive carrier mothers to their babies (129,130) and from HBeAg-positive patients to health care workers who sustain needle stick injuries (127).

During acute HBV infection, HBeAg appears shortly after the appearance of HBsAg. In patients who recover,

HBeAg to anti-HBe seroconversion precedes that of HBsAg to anti-HBs seroconversion (105) (Fig. 29.1). Anti-HBe may persist for many years after resolution of acute hepatitis B. In patients with chronic HBV infection, HBeAg may persist for years to decades (Fig. 29.2). During the HBeAg-positive phase, most patients have detectable HBV DNA in serum and active liver disease. In patients with perinatally acquired HBV infection, there may be an immune tolerant phase with normal ALT levels and minimal inflammation in the liver (131,132,133). Seroconversion from HBeAg to anti-HBe is usually associated with marked decrease in serum HBV DNA level and remission of liver disease (134,135,136). However, some anti-HBe–positive patients continue to have active liver disease and detectable HBV DNA in serum (128,137). This may be due to low levels of wild type HBV or the presence of precore HBV variants (138).

Tests for Hepatitis B Virus Deoxyribonucleic Acid in Serum

Assays for HBV DNA polymerase activity were developed in the 1970s to directly assess and quantify HBV replication (139). These assays are cumbersome and have been superseded by assays to detect HBV DNA. Serum HBV DNA levels can be quantified by molecular hybridization or signal amplification assays, which have sensitivity limits of 10^5 to 10^6 viral copies/mL. PCR assays are more sensitive and are capable of detecting less than 10^2 copies/mL (140). An arbitrary value of greater than 10^5 copies/mL has been chosen as a diagnostic criterion for chronic hepatitis B (141). However, there are problems with this definition. First, assays for HBV DNA quantification are not well standardized (Table 29.5) (142,143,144,145,146). In the last few years, there has been concerted effort to mandate standardization of all HBV DNA assays against WHO standards and to report results in international units (147,148), but this process of standardization has not been yet been implemented worldwide. Second, some patients with chronic hepatitis B have fluctuating HBV DNA levels that may at times fall below 10^5 copies/mL (149,150,151). Therefore, a single HBV DNA level on a random occasion may not be accurate in assessing HBV replicative status and need for antiviral therapy in individual patients. Third, the threshold HBV DNA level that is associated with progressive liver disease is unknown.

Table 29.5. Comparison of Hepatitis B Virus (HBV) DNA Quantification Assays

Assay (manufacturer)	Volume of sample	Sensitivity[a]		Linearity (copies/mL)
		pg/mL	Copies/mL	
Branched DNA (Bayer)	10 µL	0.002	2×10^3	3×10^3–8×10^8
Hybrid capture (Digene)	30 µL	0.5	1.4×10^5	2×10^5–1×10^9
	1 mL	0.02	5×10^3	5×10^3–3×10^6
PCR—Amplicor (Roche)	50 µL	0.001	4×10^2	4×10^2–1×10^7 Cobas: 10^2–10^5 Taqman: 10^2–10^{10}
Molecular Beacons	10–50 µL	—	<50	50–1×10^9

1 IU = approximately 5.6 copies/mL (depending on assay).
[a]One picogram of hepatitis B virus DNA = 283,000 copies (approximately 3×10^5 viral genome equivalents).

Using hybridization assays, HBV DNA can be detected approximately 1 week after the appearance of HBsAg in patients with acute hepatitis B (152). In rare cases, HBV DNA can be detected before HBsAg. PCR assays can detect HBV DNA earlier, up to 2 to 3 weeks before the appearance of HBsAg. Recovery from acute hepatitis B is usually accompanied by the disappearance of HBV DNA in serum as determined by hybridization or bDNA assays. However, HBV DNA may remain detectable in serum for many years if tested by PCR assays (153).

In patients with chronic HBV infection, spontaneous or treatment induced HBeAg seroconversion is usually accompanied by the disappearance of HBV DNA in serum as determined by unamplified assays but HBV DNA frequently remains detectable by PCR assays except in patients who have HBsAg seroconversion (154). The major role of serum HBV DNA assays in patients with chronic HBV infection is to assess HBV replication and candidacy for antiviral therapy. Patients with high pretreatment serum HBV DNA levels are less likely to respond to IFN therapy but pretreatment serum HBV DNA levels seem to be less important in predicting

response to nucleoside/nucleotide analogs (155,156). Tests for HBV DNA in serum are also important in assessing response to antiviral treatment.

Rarely, tests for HBV DNA in serum help to identify HBV as the etiology of liver disease in HBsAg-negative patients (157,158). This is especially important in patients with fulminant hepatitis B, who may have cleared HBsAg by the time they present (159). In patients with chronic liver disease due to occult HBV infection, most cases are due to low levels of HBV whereas a small percent may be related to HBV mutants that downregulate the production of HBsAg or produce aberrant HBsAg that cannot be detected in conventional serologic assays (160).

Table 29.6. Diagnosis of Hepatitis B Virus (HBV) Infection

	HBsAg	HBeAg	IgM anti-HBc	IgG anti-HBc	Anti-HBs	Anti-HBe	HBV DNA	Interpretation
Acute HBV	+	+	+		–		+++	Early phase
infection	–	–	+		–	+	+	Window phase
	–	–	–	+	+	+	±	Recovery phase
Chronic HBV	+	+	–	+	–	–	+++	HBeAg+ chronic hepatitis
infection	+	–	–	+	–	+	±	Inactive carrier state
	+	–	–	+	–	+	++	HBeAg– chronic hepatitis
	+	±	±	+	–	±	++	Exacerbations of chronic hepatitis

HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; IgM, immunoglobulin M; anti-HBc, hepatitis B core antibody; IgG, immunoglobulin G; anti-HBs, hepatitis B surface antibody; anti-HBe, hepatitis B e antibody.

Currently, most diagnostic laboratories use real-time PCR assays which have a wider range of linearity and are more accurate in quantification of HBV DNA. Some real-time PCR assays can also differentiate among various HBV genotypes and wild type versus HBV mutants such as precore mutation and antiviral-resistant mutations (161,162).

Diagnostic Algorithm

Diagnosis of HBV infection is shown in Table 29.6 and Figures 29.1 and 29.2.

The diagnosis of acute hepatitis B is based on the detection of HBsAg and IgM anti-HBc. During the initial phase of infection, markers of HBV replication: HBeAg and HBV DNA are present. Recovery is accompanied by the disappearance of HBV DNA, HBeAg to anti-HBe seroconversion and subsequently HBsAg to anti-HBs seroconversion. Rarely, patients may have entered the window period at the time of presentation; IgM anti-HBc is the sole marker of acute HBV infection in these patients. This situation is more common in patients with fulminant hepatitis B where virus clearance tends to be more rapid.

Past HBV infection is diagnosed by the detection of anti-HBs and anti-HBc (IgG). Immunity to HBV infection after vaccination is indicated by the presence of anti-HBs only.

The diagnosis of chronic HBV infection is based on the detection of HBsAg and IgG anti-HBc. Additional tests for HBV replication: HBeAg and serum HBV DNA should be performed to determine if the patient should be considered for antiviral therapy. Additional tests for hepatitis C and hepatitis D should also be performed to rule out superinfection with other hepatitis virus(es).

Clinical Manifestations

The spectrum of HBV infection varies from subclinical hepatitis, anicteric hepatitis, icteric hepatitis to fulminant hepatitis during the acute phase; and from inactive carrier state to chronic hepatitis, cirrhosis and HCC during the chronic phase. The clinical manifestations and outcome of HBV infection depend on the age at infection, the level of HBV replication and the immune status of the host. Perinatal or childhood infection is usually associated with few or no symptoms but a high risk of chronicity whereas adult acquired infection is usually associated with symptomatic hepatitis but a low risk of chronicity. The consensus definition and diagnostic criteria for clinical terms relating to HBV infection adopted at a recent National Institutes of Health (NIH) workshop are summarized in Table 29.7 (141).

Acute Hepatitis B Virus Infection

Approximately 70% of patients have subclinical or anicteric hepatitis during acute HBV infection, only 30% have icteric hepatitis. Symptomatic hepatitis is rare in neonates and it occurs in approximately 10% of children less than 4 years old and in approximately 30% of adults (163).

Symptoms and signs

The incubation period of acute HBV infection lasts 1 to 4 months. This period may be shorter in patients who have been exposed to a large inoculum (164). During the prodromal period, a serum sickness–like syndrome may develop. This is followed by insidious onset of constitutional symptoms including malaise, anorexia, nausea and occasionally vomiting, low-grade fever, myalgia and easy fatigability. Patients may have altered gustatory acuity and smell sensation. Some patients may experience intermittent mild to moderate right upper quadrant or midepigastric pain. In patients with icteric hepatitis, jaundice usually begins within 10 days after the onset of constitutional symptoms. Constitutional symptoms generally subside as jaundice develops. Clinical symptoms and jaundice usually disappear after 1 to 3 months but some patients may have persistent fatigue even after the ALT levels have returned to normal.

Table 29.7. Glossary of Clinical Terms Used in Hepatitis B Virus (HBV) Infection

DEFINITIONS
Chronic hepatitis B Chronic necroinflammatory disease of the liver caused by persistent infection with hepatitis B virus. Chronic hepatitis B can be subdivided into HBeAg positive and HBeAg negative chronic hepatitis B. **Inactive HBsAg carrier state** Persistent HBV infection of the liver without significant, ongoing necroinflammatory disease. **Resolved hepatitis B** Previous HBV infection without further virological, biochemical or histologic evidence of active virus infection or disease. **Acute exacerbation or flare of hepatitis B** Intermittent elevations of aminotransferase activity to more than ten times the upper limit of normal and more than twice the baseline value. **Reactivation of hepatitis B** Reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved hepatitis B. **HBeAg clearance** Loss of HBeAg in a person who was previously HBeAg positive. **HBeAg seroconversion** Loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative, associated with decrease in serum HBV DNA to $<10^5$ copies/mL. **HBeAg reversion** Reappearance of HBeAg in a person who was previously HBeAg negative, anti-HBe positive.
DIAGNOSTIC CRITERIA
Chronic hepatitis B
1. HBsAg+ >6 m 2. Serum HBV DNA $>10^5$ IU/mL (may be lower for HBeAg negative patients) 3. Persistent or intermittent elevation in ALT/AST levels 4. Liver biopsy showing chronic hepatitis (necroinflammatory score ≥4)[a]

Inactive HBsAg carrier state
1. HBsAg+ >6 m 2. HBeAg–, anti-HBe+ 3. Serum HBV DNA $<10^5$ IU/mL (usually $<10^3$ IU/mL) 4. Persistently normal ALT/AST levels 5. Liver biopsy confirms absence of significant hepatitis (necroinflammatory score <4)[a]
RESOLVED HEPATITIS B
1. Previous known history of acute or chronic hepatitis B or the presence of anti-HBc ± anti-HBs 2. HBsAg– 3. Undetectable serum HBV DNA[b] 4. Normal ALT levels

[a]Optional.
[b]Very low levels may be detectable using sensitive PCR assays.
HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBc, hepatitis B core; anti-HBs, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Adapted from Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000—summary of a workshop. *Gastroenterology* 2001;120(7):1828–1853.

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Physical examination can be unrevealing in many patients. The most common findings include low-grade temperature, clinical icterus, and soft mildly tender hepatomegaly. Splenomegaly may be found in approximately 5% to 15% of patients. Rarely palmar erythema or spider nevi can be detected. Mild lymph node enlargement may be present, but generalized lymphadenopathy is not a feature of acute hepatitis B.

Patients with fulminant hepatitis may present with features of hepatic encephalopathy—disturbance in sleep pattern, asterixis, mental confusion, disorientation, somnolence and coma, progressive decrease in liver span, and ascites.

Laboratory findings

Elevation of liver enzymes—alanine aminotransferase (ALT) and aspartate aminotransferase (AST)—is the

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hallmark of acute hepatitis. Values of 1,000 to 2,000 IU/L are typically seen during the acute phase. Increase in liver enzymes may precede the onset of symptoms. The ALT levels are usually higher than the AST levels. In patients with icteric hepatitis, increase in bilirubin levels usually lags behind increase in ALT levels. Although the peak ALT level reflects the degree of hepatocellular injury, it has no correlation with prognosis. Prothrombin time, which is a measure of liver derived clotting factors II, VII, IX, and X, is the best indicator of prognosis in patients with acute hepatitis. Because of the short half-life of these clotting factors (6 hours for factor VII), prothrombin time reflects the instantaneous synthetic function of the liver and the mass of viable hepatocytes. Serum albumin level is not a good marker of liver function during acute hepatitis because of its long half-life (21 days). Mild leukopenia with relative lymphocytosis is a common finding. Although red cell survival is slightly shortened in acute hepatitis, hemoglobin and hematocrit are usually within normal limits. Rare hematologic findings include hemolytic anemia associated with glucose-6-phosphatase deficiency (165) and aplastic anemia (166). In patients who recover, ALT levels usually return to normal values after 1 to 4 months, followed by normalization in bilirubin levels. Persistent elevation of ALT levels for more than 6 months suggests chronic liver injury and persistent infection.

Histologic findings

Liver biopsy is seldom indicated in acute hepatitis. Histologic changes of acute hepatitis include lobular disarray, acidophilic degeneration of hepatocytes, focal lobular necrosis, disruption of bile canaliculi with cholestasis, portal and parenchymal infiltration of inflammatory cells, as well as hypertrophy and hyperplasia of Kupffer cells and macrophages. Inflammatory infiltrates are predominantly lymphocytes and macrophages, with occasional eosinophils and neutrophils, but rarely plasma cells. In patients with severe hepatitis, hepatocyte necrosis is more extensive leading to bridging or linking up of necrotic areas in adjacent lobules. Resolution of hepatitis is signified by reduction of inflammatory infiltrates and parenchymal cell regeneration. In some cases of subfulminant hepatitis, liver biopsy may be indicated to assess the extent of liver necrosis and the need for a liver transplantation.

Sequelae

The risk of chronicity is inversely proportional to the age at infection. Less than 5% of immunocompetent adults with acute HBV infection progress to chronic infection, but up to 90% of those infected during infancy will develop chronic infection (5,10,11,12). Acute HBV infection is estimated to account for 35% to 70% of all virally related cases of fulminant hepatitis (167,168,169) but only 0.1% to 0.5% of acute hepatitis B runs a fulminant course (167,170). Mortality from fulminant hepatitis B is high, approaching 80%, unless liver transplantation can be performed. Contrary to transplantation for HBV-cirrhosis, reinfection after liver transplantation for fulminant hepatitis B is uncommon.

Chronic Hepatitis B Infection

Symptoms and signs

In areas of low or intermediate prevalence, approximately 30% to 50% of patients with chronic HBV infection have a history of classical acute hepatitis that progressed to chronic infection (171,172). The remaining 50% to 70% of patients with chronic HBV infection in these areas and most of those in high prevalence areas (predominantly perinatal infection) have no prior history of acute hepatitis. Many patients with chronic HBV infection are asymptomatic while others have nonspecific symptoms such as fatigue. Occasionally mild right upper quadrant or midepigastric pain may be present. Patients with chronic HBV infection may experience exacerbations that may be asymptomatic or mimic acute hepatitis with fatigue, anorexia, nausea and jaundice and in rare instances hepatic decompensation.

Physical examination may be unrevealing or there may be stigmata of chronic liver disease such as spider angioma and palmar erythema, and a mild hepatomegaly. In patients with cirrhosis, additional findings such as splenomegaly may be present. As the liver disease advances, hepatic decompensation may develop manifesting as variceal bleeding, ascites, peripheral edema, jaundice, and hepatic encephalopathy.

Laboratory findings

Laboratory tests can be entirely normal even in patients with well-compensated cirrhosis. Mild to moderate liver enzyme elevation may be the only biochemical abnormality in many patients with chronic hepatitis B. ALT levels may range from normal to fivefold elevated and are generally higher than AST levels, except in patients who have progressed to cirrhosis. Very high ALT levels, up to 1,000 IU/L, may be seen during exacerbations. Markers of impaired hepatic synthetic function may be observed during the exacerbations, especially in patients with underlying cirrhosis. In addition, increase in α-fetoprotein (AFP) levels, up to 1,000 ng/mL, may be present (173). The AFP levels tend to parallel or follow the ALT levels. Progression to cirrhosis is suspected when platelet count is decreased, when there is hypoalbuminemia, hyperbilirubinemia and prolongation in prothrombin time, and when AST/ALT ratio is greater than 1.

Histologic findings

Liver biopsy is useful in assessing the severity of liver damage, in predicting prognosis and in monitoring response to treatment. However, it must be recognized that liver histology can improve significantly in patients who have sustained response to antiviral therapy or spontaneous HBeAg seroconversion. Liver histology also can worsen rapidly in patients during exacerbations of hepatitis.

The predominant histologic findings include inflammatory cell infiltration in the portal tracts and periportal necrosis. The inflammatory infiltrate consists mainly of mononuclear cells. Periportal necrosis may be mild or severe leading to disruption of the limiting plate (piecemeal necrosis or interface hepatitis). As the liver damage progresses, fibrous tissue is deposited initially within the portal tracts, later extending into the centrilobular areas and adjacent portal tracts forming bridging fibrosis and eventually cirrhosis. In some patients, ground glass hepatocytes that stain positive for HBsAg can be found. Recent studies showed that these cells are found in association with retention of HBsAg (174). Traditionally, the histology of chronic hepatitis B is divided into chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), and cirrhosis (175). CPH represents a milder form of liver injury with limitation of chronic inflammatory infiltrates to the portal tracts whereas CAH represents a more severe form of liver injury characterized by the presence of piecemeal necrosis. A third form of liver injury had been described—chronic lobular hepatitis (CLH) (176). CLH is characterized by spotty necrosis and inflammation within the lobules with minimal or mild portal inflammation. It is most often seen during exacerbations of chronic hepatitis B. CPH and CAH were thought to represent two dichotomous reactions to chronic HBV infection with different prognosis. The advent of antiviral therapy and the availability of serial liver biopsies before and after spontaneous or treatment induced HBeAg seroconversion revealed that patients may progress from CPH to CAH and vice versa suggesting that these two forms of liver injury may be seen during different phases of chronic HBV infection in the same patient. To provide more objective assessment of liver injury, several numerical scoring systems have been established to permit statistical comparisons of necroinflammatory activity and fibrosis (177,178,179). An international panel recommended that the histologic diagnosis include the etiology of hepatitis, the grade of necroinflammatory activity, and the stage (extent of fibrosis) of the liver disease (180).

Immunohistochemical staining reveals the presence of HBsAg in patients with chronic HBV infection. The distribution of HBsAg can be either membranous or cytoplasmic. In patients with high levels of HBV replication, HBcAg can also be demonstrated. The distribution of HBcAg is usually nuclear but it has been observed that the distribution is shifted to the cytoplasm in patients with exacerbations or active liver disease (181).

Extrahepatic Manifestations

Extrahepatic manifestations have been reported in patients with both acute and chronic HBV infection. Extrahepatic manifestations are more commonly associated with acute hepatitis B than other forms of acute viral hepatitis and may be present in approximately 10% to 20% of patients with chronic HBV infection. They are believed to be mediated by circulating immune complexes, the formation of which is favored by high levels of HBV replication (182,183).

Serum sickness

Acute hepatitis B is sometimes heralded by a serum sickness–like syndrome manifested as fever, skin rash, polyarthralgia, and arthritis. Skin and joint manifestations usually subside rapidly with the onset of jaundice.

Polyarteritis nodosa

Approximately 10% to 50% of patients with polyarteritis nodosa (PAN) are found to be HBsAg positive. The decline in HBV infection over the past decade, especially in developed countries has also been associated with a decrease in frequency of HBV-related PAN (183,184). Immune complexes involving HBV antigens and antibodies are believed to trigger the vascular injury (185). Vasculitis may affect large, medium and small sized vessels in multiple organs including cardiovascular (pericarditis, hypertension, cardiac failure), renal (hematuria, proteinuria), gastrointestinal (abdominal pain, mesenteric vasculitis), musculoskeletal (arthralgia, arthritis), neurologic (mononeuritis, central nervous system involvement) and dermatologic (rashes) systems. The course is highly variable. Gastrointestinal involvement, especially perforation and bleeding, are the most severe manifestations and can be fatal (186). For many years, HBV-related PAN had been treated similar to non–virus-related PAN. Despite combination treatment with corticosteroid, immunosuppressive drugs and plasma exchange, mortality is high: 20% to 45% in 5 years and the outcome appears to be poorer than in non–virus-related PAN (184,187). The fact that HBV-related PAN is related to virus-mediated immune complexes suggests that therapy should be directed against HBV itself. Case reports and small case series have suggested a possible role of IFN therapy alone or in combination with plasma exchange (188,189,190), nucleoside analogs (191) or corticosteroids (192), as well as prednisone followed by lamivudine (193).

Glomerulonephritis

HBV-related glomerulonephritis is more often found in children. Membranous glomerulonephritis is most common especially among children but membranoproliferative glomerulonephritis, mesangiocapillary and focal glomerulonephritis, minimal change disease and IgA nephropathy have also been reported (194). Immune complexes of hepatitis B surface, core, and e antigens and antibodies together with complement components have been demonstrated in glomerular basement membrane and mesangium (195). Liver disease tends to be mild in patients who present with HBV-related glomerulonephritis (196). Severity of the renal disease does not correlate with the severity of the liver disease or the level of HBV replication.

Approximately 30% to 60% of children with HBV-related membranous glomerulonephritis undergo spontaneous remission. Disease remission is especially evident after HBeAg seroconversion (197,198). A significant percent of adults (30%) may progress to renal failure and as many as 10% will require maintenance dialysis (199,200). Corticosteroids are usually ineffective for treatment of HBV-related glomerulonephritis and may potentiate HBV replication (201). IFN has been reported to induce remission of HBV-related renal disease in small clinical trials (202,203) but the response has been poor especially in Asians. In Western countries, response to IFN has been more favorable in adults, especially among patients with membranous glomerulonephritis (204). Improvement of renal disease has also been reported with lamivudine (205). Recent reports suggest that the incidence of HBV-related glomerulonephritis in children has been decreasing since the implementation of the HBV vaccination programs (206,207).

Essential mixed cryoglobulinemia

Mixed cryoglobulinemia is a systemic disease involving mainly small vessels presenting as glomerulonephritis, arthritis and purpura. HBsAg, anti-HBs and HBV-like particles have been demonstrated in cryoprecipitates (208) but recent studies questioned the association between chronic HBV infection and essential mixed cryoglobulinemia.

Papular acrodermatitis (Gianotti-Crosti disease)

Papular acrodermatitis is found to be strongly associated with HBs antigenemia in children particularly

among those under the age of 4 (209,210). Circulating HBsAg and anti-HBs immune complexes are thought to play a role in the pathogenesis (209). It manifests as symmetrical, erythematous, maculopapular, nonitchy eruptions over the face, buttocks, limbs and occasionally the trunk lasting for 15 to 20 days. Mucous membranes are spared. Lymphadenopathy particularly in the axillary and inguinal regions is common. Evidence of acute hepatitis may coincide with the onset of the skin eruption or, more commonly, begins as the dermatitis starts to wane.

Aplastic anemia

Isolated cases of severe aplastic anemia occurring in the early phase of acute hepatitis have been reported (166). However, a recent study suggested that most hepatitis associated aplastic anemia are not related to hepatitis virus but mediated by immunopathologic mechanisms (211).

Special Patient Groups

Pediatric patients

HBV infection remains the most important cause of chronic hepatitis in pediatric patients. The clinical manifestation of chronic HBV infection in children is dependent on the age at infection. Perinatal HBV infection results in a high rate (90%) of chronic infection and a prolonged replicative phase. Children with perinatally acquired HBV infection are usually asymptomatic with normal ALT values despite high serum HBV DNA levels.

Acute HBV infection has been estimated to account for 10% to 25% of all cases of childhood acute hepatitis (212,213). Extrahepatic manifestations including arthralgia, arthritis, skin rash and Gianotti's papular acrodermatitis are common and have been reported in 25% of patients (213).

Approximately 15% to 30% of children with chronic HBV infection, who were infected during early childhood are symptomatic with elevated ALT levels and have chronic hepatitis on liver biopsies (214,215). These children have higher rates of spontaneous HBsAg and HBeAg seroconversion: approximately 2% and 15% to 20% per year, respectively (215) compared to children who were infected perinatally. Loss of HBsAg is uncommon (216,217,218,219).

Children with chronic HBV infection are more likely than adults to develop HBV-related glomerulonephritis. Progression of disease activity over time can be seen in approximately 50% of children (216,217), cirrhosis is uncommon but has been demonstrated in approximately 3.5% of children with chronic HBV infection at the time of presentation (214,220). Cirrhosis appears to be more frequent in boys and in those with a history of acute hepatitis (220). HCC has also been reported among children with chronic HBV infection (216,221). It is more common among Asian children, and in children with cirrhosis or a family history of HCC. Therefore, regular follow-up is important even in asymptomatic carrier children in these settings.

HBV related fulminant hepatitis is extremely rare in children. Most reported cases occurred in infants born to HBeAg-negative mothers (222,223). In a case series in Taiwan, 65% of 17 cases of childhood fulminant hepatitis were caused by HBV infection (224).

Immunocompromised patients

The clinical manifestations and natural course of chronic HBV infection in immunocompromised patients may be different from that in immunocompetent patients because of enhanced HBV replication and weak immune response.

Immunosuppressive therapy can increase HBV replication directly by stimulating the glucocorticoid responsive element in the enhancer region of the HBV genome (225) or indirectly by diminishing immune clearance. Abrupt withdrawal of immunosuppressive therapy as in cyclical chemotherapy or rapid tapering of steroid treatment has been reported to be associated with exacerbations of liver disease in HBsAg carriers as well as in immune individuals (226). These exacerbations are believed to be due to massive lysis of infected hepatocytes as the immune system recovers. Although most of the exacerbations are asymptomatic, fatal hepatic decompensation has been reported (227).

Patients with chronic renal failure on hemodialysis have an increased risk of HBV infection (228,229). Dialysis patients are usually HBeAg and serum HBV DNA positive but have no symptoms, normal ALT levels and minimal liver damage on liver biopsies (230). The clinical course of postrenal transplantation is, however, very different, with exacerbations, rapid progression to cirrhosis and an increased risk of HCC and death from liver failure (231,232,233,234,235). Recent reports showed that preemptive treatment with lamivudine decreased the risk of reactivation of hepatitis B post transplantation (236,237). In addition, lamivudine has been reported to be effective in treating hepatitic flares and hepatic decompensation due to reactivation of hepatitis B after renal transplantation (236,238). The American Society of Nephrology recommended that HBsAg-positive renal allograft recipients should receive lamivudine beginning at the time of transplantation and continuing for at least 18 to 24 months (239).

Patients with HIV infection have a high prevalence of HBV infection. This is probably related to the similarities in the mode of transmission of HBV and HIV. Patients who are coinfected with HBV and HIV

tend to have higher serum HBV DNA levels, lower ALT levels, lower rate of spontaneous as well as treatment related HBeAg seroconversion, and higher risk of cirrhosis (240,241,242,243). Reactivation of HBV replication has been described in association with HIV infection (244) and may lead to acceleration of liver disease progression (245). The response to HBV vaccination in HIV infected patients is also impaired (246). In contrast, HBV coinfection does not appear to have any significant effect on the rate of progression of HIV disease (242,247). More recently, severe cases of HBV disease exacerbation and deaths due to liver failure are being increasingly reported in patients receiving highly active antiretroviral therapy (HAART). The exacerbations are felt to be related to "immune reconstitution" with subsequent immune-mediated injury directed against infected hepatocytes (245,248). Several studies have shown that patients coinfected with HIV and HBV have increased risk of liver-related mortality compared to those with HIV or HBV monoinfection (249,250).

Natural History

The natural course of HBV infection is determined by the interplay between the virus: HBV replication, HBV genotype and viral variants; host: Age, gender, race/ethnicity, genetic make-up, and immune response; and environment: Alcohol, concomitant infection with other viruses—hepatitis C, hepatitis D, human immunodeficiency virus (HCV, HDV, HIV), and carcinogens such as aflatoxin.

Progression from Acute to Chronic Hepatitis B Virus Infection

The overall rate of progression from acute to chronic HBV infection has been estimated to be 5% to 10%. The risk is inversely proportional to the age at infection: 90% for perinatal infection, 20% for childhood infection, and less than 5% for adult infection (5,10,11,12). Careful analyses of patients presenting with "acute hepatitis B" found that the risk of progression to chronic HBV infection among immunocompetent adults was less than 1% after exclusion of patients who had acute exacerbations of chronic HBV infection.

Hepatitis B Virus Infection is a Life-Long Infection

The advent of sensitive molecular virology assays has revolutionized the concept of viral clearance and recovery from HBV infection. Many studies found that HBV DNA and vigorous immune response to HBV antigens can be detected more than 10 years after recovery from acute HBV infection—HBsAg to anti-HBs seroconversion (251,252). These findings indicate that HBV persists but is contained by the host immune response. This accounts for reports of chemotherapy induced reactivation of HBV replication in patients with "recovered" HBV infection (226).

The likelihood of spontaneous viral clearance in patients with chronic HBV infection is very low because

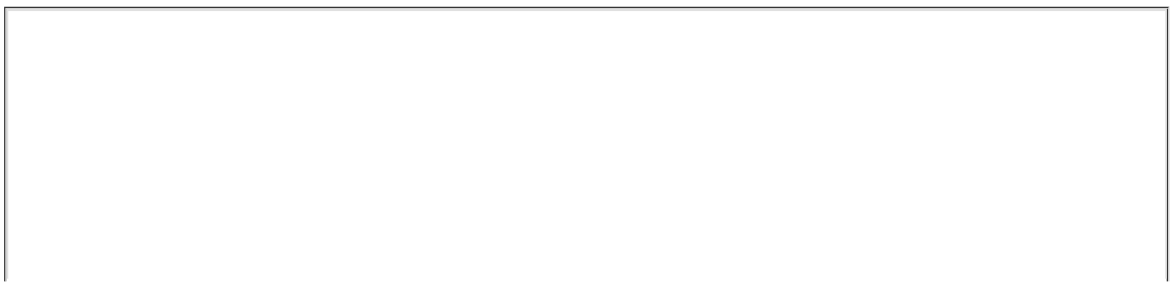
of the presence of extrahepatic reservoirs of HBV, integration of HBV DNA into the host genome, and the presence of an intracellular conversion pathway whereby newly replicated HBV DNA re-enters the hepatocyte nuclei and is used to amplify covalently closed circular HBV DNA (cccDNA) (253). This intracellular pathway enables the establishment of a pool of transcriptional templates in the hepatocyte without the need for multiple rounds of reinfection. Therefore, spontaneous viral clearance is unlikely to occur once chronic HBV infection is established.

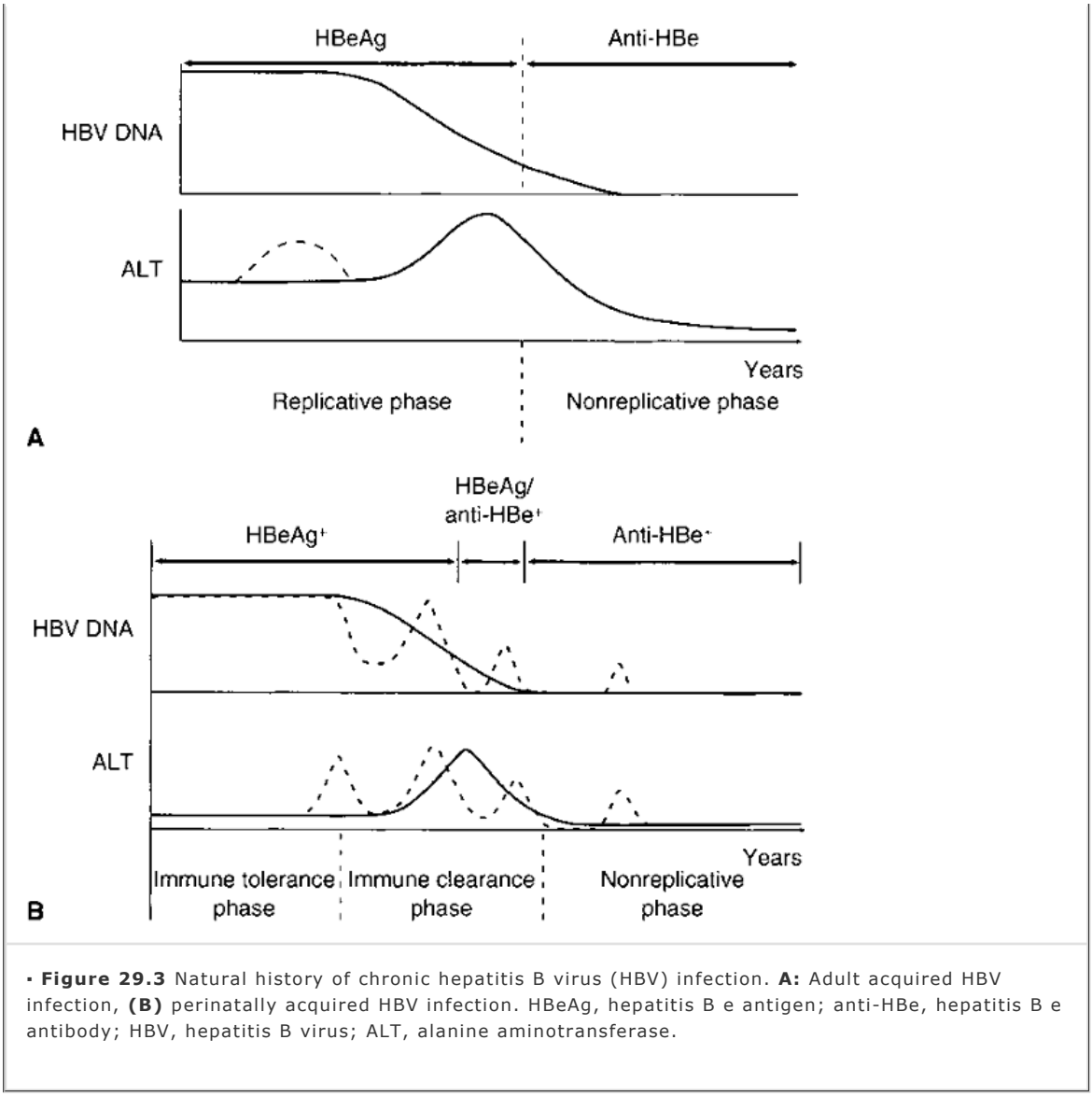
Clinical Course of Chronic Hepatitis B Virus Infection

The natural course of chronic HBV infection is characterized by fluctuations in level of HBV replication and activity of liver disease. The clinical course of chronic HBV infection can be considered as comprising four phases (Fig. 29.3) although not all patients go through every phase.

Immune-tolerant phase

In patients with perinatally acquired HBV infection, the initial phase is characterized by high levels of HBV replication: Presence of HBeAg and high levels of HBV DNA in serum (10^{6-10} IU/mL), normal ALT and minimal changes on liver biopsy (131,133,254). A mild degree of liver injury despite high levels of HBV replication is believed to be due to immune tolerance to HBV. The exact mechanism(s) for immune tolerance is unknown. Experiments in mice suggest that transplacental transfer of maternal HBeAg may induce a specific unresponsiveness of helper T cells to HBeAg (255). Because HBeAg and HBcAg are cross-reactive at the T-cell level, deletion of T-helper cell response to HBeAg results in ineffective cytotoxic T-cell response to HBcAg (256).





· Figure 29.3 Natural history of chronic hepatitis B virus (HBV) infection. **A:** Adult acquired HBV infection, **(B)** perinatally acquired HBV infection. HBeAg, hepatitis B e antigen; anti-HBe, hepatitis B e antibody; HBV, hepatitis B virus; ALT, alanine aminotransferase.

During the immune tolerance phase, which lasts 1 to 4 decades, there is a very low rate of spontaneous HBeAg clearance. The cumulative rate of spontaneous HBeAg clearance is estimated to be approximately 2% during the first 3 years (133,257) and only 15% after 20 years of infection (258). Persistence of high levels of viremia in adolescents and young adults accounts for the high frequency of maternal–infant transmission of HBV in Asia. The lack of assistance from immune-mediated viral clearance also contributes to a low rate of treatment-related HBeAg seroconversion.

A study from Taiwan followed 240 adult patients (54% men, mean age 28 years) who presented during this phase and found that only 5% progressed to cirrhosis and none to HCC during a mean follow-up of 10.5 years (259). The risk of cirrhosis was higher in

patients who had HBeAg seroconversion at an older age and those who had relapse of hepatitis after HBeAg seroconversion. These findings indicate that presence of HBeAg at presentation is not invariably associated with high risks of cirrhosis and HCC, rather the risk of adverse clinical outcome is related to a long duration of high levels of HBV replication and active hepatitis.

In patients with childhood or adult acquired HBV infection, the "immune-tolerant" phase is short-lived or absent.

Immune clearance phase/hepatitis B e antigen–positive chronic hepatitis

This phase is characterized by the presence of HBeAg, high levels of serum HBV DNA and active liver disease (elevated ALT and necroinflammation on liver biopsy). In patients with perinatally acquired HBV infection, transition from the immune tolerant to the immune clearance phase usually occurs during the second to fourth decades of life. Most patients with childhood or adult acquired HBV infection are already

in the immune clearance phase at presentation.

During this phase, spontaneous HBeAg clearance occurs at an annual rate of 10% to 20% (258,259,260) (Fig. 29.3). HBeAg seroconversion is frequently but not always accompanied by biochemical exacerbations (215,258,261). These exacerbations are believed to be due to a sudden increase in immune-mediated lysis of infected hepatocytes, and are often preceded by an increase in serum HBV DNA level (262) and a change in distribution of HBcAg from nuclear to cytoplasmic localization in the hepatocytes (181).

Most exacerbations are asymptomatic but some are accompanied by symptoms of acute hepatitis. Occasionally, IgM anti-HBc may be detected leading to misdiagnosis of acute hepatitis B in previously unrecognized carriers (117). Exacerbations may be associated with increase in α-fetoprotein levels (173,260). In approximately 2.5% of patients (especially those with preexisting cirrhosis), exacerbations may result in hepatic decompensation and rarely death from hepatic failure (263).

Not all exacerbations lead to HBeAg seroconversion (258,264,265). Some patients have suboptimal immune response and abortive immune clearance. These patients may develop recurrent exacerbations with intermittently undetectable serum HBV DNA with or without transient loss of HBeAg. Repeated episodes of necroinflammation may increase the risk of cirrhosis and HCC. Exacerbations are more commonly observed in men than in women (265) and may account for a higher incidence of HBV-related cirrhosis and HCC among men.

An important outcome of the "immune clearance" phase is HBeAg to anti-HBe seroconversion. Factors associated with higher rates of spontaneous HBeAg seroconversion include older age, higher ALT levels and more recently HBV genotype B (258,260,266,267,268,269,270). High ALT level is believed to be a reflection of vigorous host immune response accounting for its strong correlation with spontaneous as well as treatment-related HBeAg seroconversion. Studies from Asian countries where genotypes B and C predominate showed that HBV genotype B is associated with a lower prevalence of HBeAg, HBeAg seroconversion at an earlier age, and more sustained virologic and biochemical remission after HBeAg seroconversion (266,270).

Inactive carrier phase

This phase is characterized by absence of HBeAg, presence of anti-HBe, persistently normal ALT levels, and low or undetectable serum HBV DNA (usually $<10^3$ IU/mL) (141). Liver biopsy generally shows mild hepatitis and minimal fibrosis but inactive cirrhosis may be observed in patients who had accrued substantial liver injury during the preceding "immune clearance" phase.

The inactive carrier phase may persist indefinitely, in which case the prognosis is generally favorable especially if this phase is reached early. This is supported by the finding of comparable survival between HBsAg-positive blood donors (almost all were HBeAg negative and had normal ALT at baseline) and uninfected controls over a 30-year period (271).

Some patients in the inactive carrier phase eventually clear HBsAg. The annual rate of HBsAg clearance has been estimated to be 0.5% to 2% (267,272). Despite HBsAg clearance, some patients have residual liver disease and some may develop HCC, the risk of HCC is higher in those who have progressed to cirrhosis prior to HBsAg clearance (273,274,275).

Some inactive carriers have reactivation of HBV replication later in life. Reactivation may occur spontaneously or as a result of immunosuppression, and may be due to wild type HBV or HBV variants that abolish or downregulate HBeAg production. In one study of 283 Chinese patients followed for a median of 8.6 years after spontaneous HBeAg seroconversion, 67% had sustained remission, 4% had HBeAg reversion and 24% had HBeAg-negative chronic hepatitis (276). Cirrhosis developed in 8% and HCC in 2%, the risk being higher in those who had active hepatitis after HBeAg seroconversion.

Reactivation of hepatitis B virus replication/hepatitis B e antigen–negative chronic hepatitis

This phase is characterized by absence of HBeAg, presence of anti-HBe, detectable serum HBV DNA, elevated ALT, and chronic inflammation ± fibrosis on biopsy (Fig. 29.3) (141,277).

Patients in this phase are usually older and have more advanced liver disease because this represents a later phase in the course of chronic HBV infection. Serum HBV DNA levels are lower than in HBeAg-positive patients but may reach 10^{8-9} IU/mL.

The hallmark of this phase is its fluctuating course. In one study where patients were monitored at monthly intervals, 36% had persistently elevated ALT, 20% had fluctuating abnormal ALT, and 44% had fluctuating ALT with intermittently normal values (278). Several investigators have attempted to define cutoff HBV DNA levels that would differentiate patients with HBeAg-negative chronic hepatitis from inactive carriers; because of the fluctuating course, serial testing is more reliable than any single HBV DNA level at a random time point (213,215,279).

HBeAg-negative chronic hepatitis was originally reported in Mediterranean countries. It has now been reported in all parts of the world (280). The geographic variations in prevalence of HBeAg-negative chronic

hepatitis are related to the predominant HBV genotype in that region. Recent studies in Europe, Asia and the United States have all reported an increased prevalence of HBeAg-negative and a decreased prevalence of HBeAg-positive chronic hepatitis (281,282). This may be related to increased awareness, decrease in new HBV infections due to vaccination, and progression of the existing pool of HBV carriers to later stages of chronic HBV infection.

Latent/Occult Hepatitis B Virus Infection

Occult HBV infection is defined as the detection of HBV DNA in persons who are HBsAg negative (283). HBV DNA is more often detected in liver than in serum. It has been proposed that diagnosis of occult HBV infection be made only when HBV DNA can be detected using at least two sets of primers from different regions of the HBV genome in duplicate assays.

Prevalence of occult HBV infection is higher in countries that are endemic for HBV and in individuals with serologic markers of HBV exposure. (283). HBV-DNA detection rate is highest in subjects who are anti-HBc positive/anti-HBs negative; some of these individuals probably have low-level HBV infection with subdetectable HBsAg. The HBV-DNA detection rate is intermediate in subjects who are positive for both anti-HBc and anti-HBs. These individuals may have recovered from previous infection. The HBV-DNA detection rate is lowest in seronegative subjects. These individuals have recovered from previous HBV infection but lost all serologic markers. Rarely, they may be infected with HBV mutants that do not express HBV serologic markers.

Occult HBV infection is more common among patients with cirrhosis or HCC (284). Many of these patients probably had chronic HBV infection for decades leading to liver damage but HBsAg is no longer detectable when cirrhosis or HCC is diagnosed. Low levels of HBV may also be a cofactor of liver disease in patients with chronic HCV infection, nonalcoholic fatty liver disease, α_1-antitrypsin deficiency and other causes of chronic liver disease. Whether occult HBV infection alone can cause cirrhosis or HCC is unclear.

Sequelae of Chronic Hepatitis B Virus Infection

The sequelae of chronic HBV infection vary from inactive carrier state to chronic hepatitis, cirrhosis, hepatic decompensation, HCC and death. The clinical outcome of patients with chronic HBV infection depends on the severity of liver damage prior to sustained HBeAg seroconversion and the durability of the inactive carrier phase.

Annual rate of progression from chronic hepatitis to cirrhosis has been estimated to be 2% to 6% for HBeAg-positive and 8% to 9% for HBeAg-negative patients (171,267,285,286,287), the higher rate in HBeAg-negative patients is related to older age and more advanced liver disease at presentation. Factors that have been reported to be associated with increased rate of progression to cirrhosis include: Host (older age, men), virus (persistent high levels of HBV replication, HBV genotype [C > B], coinfection with HCV, HDV, and HIV), and environment (alcohol and more recently obesity) (267,285,287,288,289,290,291,292,293,294). One study from Taiwan found that the 10-year cumulative probability of cirrhosis among chronic hepatitis B patients with HCV, HDV, and no superinfection was 48%, 21%, and 9%, respectively (290). Several studies showed that patients who had HBeAg reversion had increased risk of cirrhosis compared to those who had sustained HBeAg seroconversion (267,276). One study reported that the adjusted relative risk of cirrhosis for patients with serum HBV DNA greater than 10^4 and greater than 10^6 copies/mL was 2.3 and 9.3 compared to carriers with serum HBV DNA less than 10^4 copies/mL (294). These data suggest that persistent high levels of HBV replication (with accompanying hepatitis) increase the risk of cirrhosis. Studies in Asia found that genotype C is associated with a more rapid rate of progression to cirrhosis than genotype B (291,292,293) possibly related to a longer duration of high levels of HBV replication and more active hepatitis.

Annual rate of progression from compensated cirrhosis to hepatic decompensation has been estimated to be 4% to 6% (287,295,296). Survival after the development of compensated cirrhosis is favorable initially (85% at 5 years) but decreases dramatically after the

onset of decompensation to 55% to 70% at 1 year and 14% to 35% at 5 years (276,295,297,298,299,300). The lifetime risk of a liver-related death was estimated to be 40% to 50% for men and 15% for women among Chinese patients with chronic HBV infection (301). Several studies revealed that persistent high level HBV replication is associated with increased mortality in patients with cirrhosis, while biochemical remission, clearance of HBeAg or HBV DNA from serum after the diagnosis of cirrhosis were associated with significantly higher rate of survival. (298,299).

Annual rate of HCC development has been estimated to be 0.5% to 1.0% for noncirrhotic carriers and 2.5% to 3% for patients with cirrhosis (171,285,295,296,297,300,302,303). Risk factors for HCC include host (older age, male gender, being Asian, and having first-degree relatives with HCC), virus (high levels of HBV replication, HBV genotype [C > B], core promoter mutations and coinfection with HCV), and environment (alcohol, aflatoxin, and more recently smoking, obesity, and diabetes) (291,292,295,296,297,300,301,303,304,305,306,307,308,309,310). It is important to note that although HCC is more common among patients with cirrhosis, 30% to 50% of HCC associated with HBV occurs in the absence of cirrhosis.

Recent studies found an association between HBV replication and the risk of HCC. In one study of 11,893 Taiwanese men aged 30 to 65 years, followed for a mean of 8.5 years, the adjusted relative risk of HCC was six- to sevenfold higher among HBsAg men who were HBeAg positive at entry than those who were HBsAg positive, HBeAg negative (306). Another study from Taiwan found that the risk of HCC increased with increasing baseline serum HBV DNA levels (307). These findings were confirmed by studies in Senegal and Mainland China (311). Unfortunately, none of these studies monitored serum HBV DNA and ALT levels over time. It is likely that the duration of high levels of HBV replication as well as the duration and severity of hepatitis activity are more important than a single high HBV DNA level on a random occasion in predicting the risk of HCC in individual carriers.

Several studies from Asia including the above study from Taiwan reported that genotype C is associated with increased risk of HCC compared to genotype B (291,292,307). This may be related to a longer duration of high levels of HBV replication and active hepatitis and a higher frequency of core promoter mutations. Core promoter mutations have been shown in some studies to be associated with increased risk of HCC and to precede HCC diagnosis (308,309,312). Core promoter mutations have been found to be associated with more active hepatitis and the most common mutations ($A_{1762}T$, $G_{1764}A$) result in corresponding changes in the overlapping *X* gene. In vitro studies found that the HBx protein is a potent transactivator and may activate host genes including oncogenes (313).

Coinfection of Hepatitis B Virus and Hepatitis C Virus

Acute coinfection of HBV and HCV has been reported in transfused patients as well as in intravenous drug users (314,315). Coinfection with HCV may delay the onset and shorten the duration of HBs antigenemia as well as lower the peak ALT levels compared with acute HBV infection alone (314). These findings suggest that HCV coinfection may interfere with the replication of HBV leading to attenuation of liver damage. However, acute coinfection of HCV and HBV has been reported to increase the risk of fulminant hepatic failure (316). HCV superinfection in HBsAg carriers typically manifests as acute icteric hepatitis and is associated with a high risk of hepatic decompensation and death from hepatic failure (290). HCV superinfection has also been reported to decrease HBV DNA levels and to be associated with earlier HBsAg clearance (317,318,319,320). Most patients coinfected with HCV and HBV have detectable serum HCV RNA but not HBeAg or HBV DNA indicating that HCV infection is dominant. Nevertheless, liver disease is usually more severe and the risks of cirrhosis and HCC are higher compared to patients infected with either virus alone (321,322).

Hepatitis B Virus and Hepatocellular Carcinoma

Worldwide, HCC is the third most common cause of cancer deaths in men and the fifth most common cause of cancer deaths in women accounting for approximately 500,000 deaths each year (323). The vast majority (85%) of HCC is concentrated in eastern and southeastern Asia and sub-Saharan Africa (324) where HBV infection is endemic. Several lines of evidence support an etiologic association between chronic HBV infection and HCC.

Epidemiologic Association

There is a close correlation between the geographic distribution of HBsAg carriers and the occurrence of HCC (325,326,327). A high proportion of patients with HCC have chronic HBV infection, and HBV infection precedes HCC. The strongest evidence for an etiologic association between chronic HBV infection and the development of HCC is derived from a study of 22,707 Taiwanese men who were followed for a mean of 8.9 years (301). The incidence of HCC was 495/100,000

per year for HBsAg positive and 5/100,000 per year for HBsAg-negative men, with a relative risk of 98. The incidence of HCC/100,000 per year among HBsAg-negative men was 10, 5, and 0 for individuals who were anti-HBc alone positive, anti-HBc and anti-HBs positive, and seronegative, respectively.

Animal Models

Chronic infection with other hepadnaviruses has also been shown to be associated with the development of HCC. In one study, virtually 100% of the woodchucks chronically infected with woodchuck hepatitis virus (WHV) developed HCC 2 to 4 years after the onset of infection (328). These woodchucks were bred and raised in captivity and were fed strictly regulated food free of aflatoxin and other carcinogens. In the same study, only 6.5% of woodchucks had recovered from transient WHV infection and none of the uninfected woodchucks developed HCC. These data suggest that chronic infection with hepadnavirus alone is sufficient to cause HCC.

Physical Presence

The physical presence of HBV in HCC has been demonstrated by the finding of integrated HBV DNA in neoplastic liver tissues from most of the HBsAg-positive patients as well as some HBsAg-negative patients (329,330). In most instances, HBV DNA is present as a discrete high-molecular-weight band suggesting clonal expansion of hepatocytes that contain integrated HBV DNA. It should be noted that integrated HBV DNA can also be detected in adjacent non-neoplastic liver tissues as well as in liver tissues of patients with

HBV-related chronic hepatitis or cirrhosis (330,331).

Prevention of Hepatocellular Carcinoma Through Vaccination and Antiviral Therapy

Recent studies demonstrating that HBV-related HCC can be prevented through HBV vaccination and antiviral therapy provided further support of the etiologic association. Studies from Taiwan reported a significant decrease in the incidence of childhood HCCs that accompanied the decline in prevalence of chronic HBV infection among Taiwanese children after the implementation of universal vaccination of newborns (77).

Several follow-up studies of chronic hepatitis B patients who received IFN or lamivudine therapy observed a decrease in incidence of HCC among patients with long-term virologic response. The best evidence that antiviral treatment can prevent HCC was provided by a prospective double blind placebo-controlled trial of lamivudine treatment in patients with high levels of HBV replication and bridging fibrosis or cirrhosis. After a median duration of 32 months, a significantly lower percent of treated patients had HCC (332). The efficacy of antiviral therapy in preventing HCC was also demonstrated in woodchucks that received lamivudine, clevudine and entecavir for chronic WHV infection (333,334).

Mechanisms of Hepatocarcinogenesis

Chronic HBV infection can induce HCC directly by activating cellular oncogenes or by inactivating tumor suppressor genes, or indirectly through chronic liver injury, inflammation, and regeneration (335).

To date there is no evidence that HBV DNA is directly oncogenic. Transfection of HBV DNA has not been demonstrated to induce malignant transformation of cultured cells. Analyses of integrated HBV DNA in neoplastic tissues from patients with HCC revealed varying portions of the HBV genome with deletions and duplications (335,336). An intact region of the HBV genome that is universally incorporated has not been identified. The long latent interval between the onset of HBV infection and the development of HCC also make it unlikely that HBV is an oncogenic virus.

Integration of HBV DNA may activate cellular proto-oncogenes or suppress growth-regulating genes. Recent studies found that HBV DNA integration frequently targets genes that regulate key cellular pathways such as calcium homeostasis, mean arterial pressure (MAP) kinase–dependent signaling pathways, and the telomerase gene (335,337).

Integration of HBV DNA can also induce carcinogenesis via transactivation. The HBx protein has been shown to be a potent and promiscuous transactivator of viral as well as cellular enhancer and promoter (313,338). Integration of the intact or truncated versions of the *X* gene is found in most HCCs. These sequences retain their transactivating capacity and may activate cellular oncogenes (339). It has also been reported that HBx protein binds the tumor suppressor gene p53 leading to decreased transcription and reduced cellular growth inhibition (340,341). Moreover, HBx protein has been shown to regulate proteasome function and to have an effect on calcium homeostasis and mitochondrial function (335). Finally, mutations in amino acids 130 and 131 of the X protein which corresponds to the most common core promoter mutations have been found in many patients with HCC and to precede HCC development (312). Another viral protein that may play a role in hepatocarcinogenesis is the 3′-truncated preS/S sequence which is frequently found in HCCs (342). More recently, a spliced HBV transcript and its encoded protein (HBSP) has also

been incriminated as playing a role in HCC development through its effect on HBV replication and liver fibrosis (343).

Integration of HBV DNA can also cause cancers indirectly via chromosomal deletions or translocations.

An alternate path by which chronic HBV infection leads to HCC is through induction of liver injury. Chronic liver cell injury initiates a cascade of events characterized by increased rates of cellular DNA synthesis and impaired cellular repair thereby setting the scene for acquired mutations. Mutations may be promoted by the release of inflammatory cytokines and exposure to environmental carcinogens. During regeneration, transformed cells that have a growth advantage are selected resulting in clonal expansion and eventually tumor formation. Experimental evidence for this hypothesis is derived from observations that transgenic mice which overexpress HBV large S protein develop severe hepatocellular injury followed by regenerative hyperplasia, transcriptional dysregulation, aneuploidy and finally neoplasia (174). Clinical studies support the conclusion that long durations of high levels of HBV replication and active hepatitis increase the risk of HCC.

Hepatitis B Virus Genotypes and Variants

The HBV genome consists of four partially overlapping open reading frames: The *pre-S/S* gene that codes for the envelope proteins, the *pre-C/C* gene that codes for the e antigen and core protein, the *P* gene that codes for the DNA polymerase and reverse transcriptase, and the *X* gene that codes for a protein of unclear significance. Although HBV is a DNA virus, it is prone to mutations with a rate of nucleotide substitutions estimated at 1×10^{-5} to 3×10^{-5}/site per year (344). This is related to the reverse transcription of an RNA

intermediate during the replication cycle of HBV. Mutations occur at random along the HBV genome but the overlapping open reading frames limit the number and location of viable mutations. Mutations that confer a survival advantage to the virus by enhancing replication or evading immune response tend to be selected.

Hepatitis B Virus Genotypes

HBV can be classified into eight genotypes designated A-H based on an intergroup divergence of 8% or more in the complete nucleotide sequence (106,107,345). The geographic distribution of HBV genotypes is summarized in Table 29.8. There is an association between HBV genotypes and precore and core promoter variants. The most common precore variant ($G_{1896}A$) is predominantly found in patients with HBV genotypes B, C, and D and rarely in patients with genotype A (266,346,347,348). This accounts for the high prevalence of HBeAg-negative chronic hepatitis and precore stop codon variant ($G_{1896}A$) in Asia and the Mediterranean basin and their low prevalence in the United States and Northern Europe. The most common core promoter variant ($A_{1762}T$, $G_{1764}A$) is more frequently found in patients with HBV genotypes A, C, and D (266).

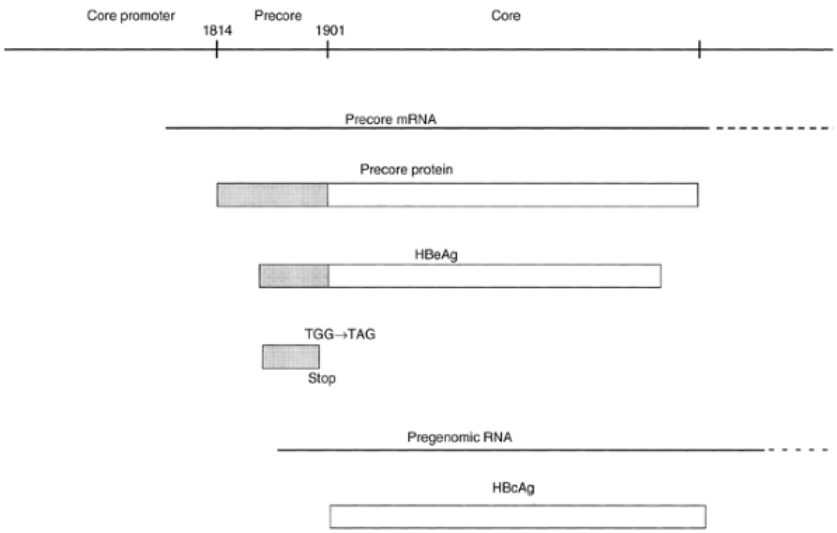
Table 29.8. Geographic Distribution of Hepatitis B Virus (HBV) Genotypes

HBV genotypes	Distribution areas
A	North-west Europe, North America, Central Africa
B	China, Japan, Indonesia, Vietnam
C	China, Japan, East Asia, Korea, Vietnam, Polynesia
D	Mediterranean basin, Middle East, India
E	Africa
F	American natives, Polynesia
G	United States, France
H	Mexico, Latin America

Several studies suggest that HBV genotypes may be related to the rate of recovery or likelihood of a fulminant course during acute infection (349,350,351,352); these studies involved small numbers of patients and the data need to be confirmed.

Studies in Asia where genotypes B and C are predominant found that genotype B is associated with a lower prevalence of HBeAg, earlier HBeAg seroconversion, higher likelihood of sustained remission after HBeAg seroconversion, and less active liver disease compared to genotype C (107,266,270,353,354). HBV genotype B is also associated with a slower rate of progression to cirrhosis and HCC (291,292,293,355,356,357). Data on the relation between other HBV genotypes and HBV replication and liver disease are scanty. Available data suggest that genotype A is associated with a higher likelihood of sustained virologic and biochemical remission after HBeAg seroconversion than genotype D (358).

HBV genotype may also impact response to IFN therapy. Several studies reported that HBeAg-positive patients with genotypes A and B have higher rates of HBeAg loss than those with genotypes C and D (359,360,361). There is as yet no evidence that HBV genotypes are related to IFN response among HBeAg-negative patients. Nucleoside/nucleotide analogs appear to be equally effective in virus suppression across all HBV genotypes (362). Data on the association between HBV genotypes and durability of response and rate of drug resistance are conflicting and limited by the small number of patients studied and heterogeneity in treatment regimen (363,364,365,366).



▪ **Figure 29.4** Transcription and translation of precore/core open reading frame. Precore mRNA is translated into precore protein which is processed at the N and C terminal ends to hepatitis B e antigen (HBeAg). Mutation of nucleotide 1896 from G to A converts codon 28 in the precore region from tryptophan to stop codon thereby preventing production of HBeAg. Pregenomic RNA is translated into core and polymerase protein and reverse transcribed into hepatitis B virus DNA. HBcAg, hepatitis B core antigen.

Precore Variants

Among the naturally occurring HBV mutations, mutations in the precore region have been most extensively studied (Fig. 29.4). The precore region consists of 87 nucleotides (29 amino acids) that precede the core region. The *preC/C* gene codes for two 3.5-kb RNA transcripts. The precore mRNA is slightly longer and initiates upstream of the pregenomic RNA. It codes for a precursor protein which includes the entire precore/core gene. The nascent precore/core protein is processed at both the N and C terminal ends giving rise to a smaller secretory protein—HBeAg (367). The pregenomic RNA serves as a template for reverse transcription into the (−) strand HBV DNA. It also serves as an mRNA for translation into the core protein and polymerase protein.

The predominant mutation in the precore region is a G-A change at nucleotide 1896, creating a stop codon at codon 28 ($G_{1896}A$, eW28X) (138) (Table 29.9). This mutation leads to premature termination of the precore/core protein, therefore preventing the production of HBeAg. Because the precore region is not essential for HBV replication and the $G_{1896}A$ mutation is upstream of the core region, HBV replication and HBcAg expression are not affected. Precore variants may be selected because of their ability to evade immune clearance or to enhance HBV replication.

Epidemiology and transmission

Initial reports of the $G_{1896}A$ variant came from the Mediterranean countries and Japan (138,368,369,370). Recent studies found that this variant can be detected in diverse geographical areas (280,371,372). Although it was previously thought to be rare in North America and Western Europe, recent studies found that precore variant can be found in up to 30% patients with chronic HBV infection in the United States (282,373,374). The variability in the prevalence of the $G_{1896}A$ variant in different countries is related to the predominant HBV genotype and the nucleotide at position 1858, located opposite 1896 in the stem-loop structure of the pregenome encapsidation sequence (ε) (371,375). The $G_{1896}A$ variant is replication competent and infectious. Transmission has been documented in humans from infected mothers to infants, between sexual partners, and nosocomially, as well as in chimpanzee experiments (369,376,377,378). However, infection with precore HBV variant is less likely to progress to chronic

infection possibly due to the lack of the tolerogenic effect of HBeAg.

Table 29.9. Common Forms Of Hepatitis B Virus (HBV) Variants/Mutants

HBeAg NEGATIVE PHENOTYPE			
Pre-core region			
G-A at nt 1896, tryptophan-stop at codon 28 (G1896A, eW28X)			
G-A at nt 1899, glycine-aspartate at codon 29 (G1899A, eG29D)			
Core promoter region			
A-T at nt 1762 and G-A at nt 1764 (A1762T, G1764A, ×K130M, ×V131I)			
HBIG/HBV VACCINE ESCAPE			
***S* Gene**			
Glycine-arginine at codon 145 (sG145R)			
ANTIVIRAL-RESISTANCE			
***P* gene (reverse transcriptase/polymerase region)**			
Lamivudine	L180M	M204V/I	
Adefovir	A181V/T	N236T	
Entecavir	T184G	S202G/I	M250V
Telbivudine		M204I	
L180M	Leucine-methionine at codon 180		
A181V/T	Alanine-valine or threonine at codon 181		
T184G	Threonine-glycine at codon 184		
S202G/I	Serine-glycine/isoleucine at codon 202		
M204V/I	Methionine-valine or isoleucine at codon 204		
N236T	Asparagine to threonine at codon 236		
M250V	Methionine to valine at codon 250		

HBeAg, hepatitis e antigen; HBIG, hepatitis B immune globulin; nt, nucleotide.

Many other mutations in the precore region have been reported including mutations of the start codon but they are less common and their clinical significance is less certain.

Pathogenesis and clinical manifestations

The $G_{1896}A$ variant is usually found in HBeAg-negative patients but may be present as a mixture with wild

type virus in HBeAg-positive patients. It has been observed to emerge or become selected as the predominant species around the time of HBeAg seroconversion (368,370,379). The $G_{1896}A$ variant was initially thought to cause more severe liver disease because it was found in patients with CAH or fulminant hepatitis (138,368,369,377,378). However, some of the studies on patients with fulminant hepatitis reported that the same mutation was also found in the source patients (369,377,378). The $G_{1896}A$ variant has also been detected in anti-HBe–positive asymptomatic carriers (370,372,380). Therefore, the $G_{1896}A$ mutation alone may have no direct pathogenic role; instead host immune response and mutations in other regions of the HBV genome may be more important in determining the severity of liver disease.

Diagnosis

The presence of the $G_{1896}AG_{1896}A$, presence of variant should be suspected in patients who are HBsAg positive, HBeAg negative, anti-HBe positive; but continue to have elevated ALT levels, active hepatitis and HBcAg expression on liver biopsies. Confirmation that the liver disease is due to persistent HBV replication should be made by detection of HBV DNA in serum and by excluding other causes of liver disease including superimposed hepatitis C and D. Detection of the $G_{1896}A$ variant can be achieved by many methods including direct sequencing, restriction fragment length polymorphism (RFLP), line probe assay, ligase chain reaction, colorimetric point mutation assay, and real-time PCR assay.

Treatment

Anti-HBe–positive patients who have the $G_{1896}A$ variant have been reported to have lower rates of sustained response to IFN as well as nucleoside/nucleotide treatment compared to HBeAg-positive ones (381). The role of the $G_{1896}A$ variant in response to IFN therapy in patients with HBeAg-positive chronic hepatitis is less clear. One study reported that HBeAg-positive patients who had $G_{1896}A$ variant either as the predominant species or as a mixture with wild type HBV had higher rates of response (379). It is possible that these patients

were on the way to spontaneous HBeAg seroconversion and IFN therapy merely hastened the process. There is no evidence that antiviral treatment induces a higher rate or unique pattern of mutations in the precore region (379).

Core Promoter Mutations

Mutations in the core promoter region can also prevent HBeAg production without affecting HBV replication or HBcAg expression by selectively downregulating the transcription of the precore mRNA but without affecting the pregenomic RNA (382,383). The most common core promoter variant had dual mutations involving A to T change at nucleotide 1762 and G to A change at nucleotide 1764 ($A_{1762}T$, $G_{1764}A$) (Table 29.9). These two changes result in amino acid substitutions in codons 130 and 131 of the overlapping X gene. Unlike precore variants, core promoter variants can be detected in similar proportions of HBeAg-negative and HBeAg-positive patients (281,282,384,385). One study demonstrated that emergence of the core promoter changes was more common among HBeAg-positive patients who subsequently seroconvert compared to those who remained HBeAg positive (386), indicating that the selection of the core promoter variant may play a role in HBeAg clearance. Core promoter variants are more commonly found in patients with CAH, fulminant hepatitis or HCC (308,387,388,389,390,391). Core promoter mutations have been shown in several studies to be associated with HCC, whether this is related to more active liver disease, frequent association with genotype C, or mutations in the overlapping *X* gene is unclear.

Core Gene Mutations

The HBV core protein is essential for virus replication. Mutations in the HBV core gene have been detected in patients with chronic HBV infection (392,393,394,395). Mutations in the core gene are more often reported in association with older age, HBeAg negativity, active liver disease and presence of the precore variant (392). Longitudinal studies found that most of the mutations occur around the time of HBeAg seroconversion or exacerbations of chronic hepatitis (396). Naturally occurring mutations involving critical residues in the HLA-A2 restricted CTL epitope (amino acids 18 to 27) leading to T-cell receptor antagonism have been reported (397). However, CTL escape mutant is not the cause of persistent infection in most of the patients with chronic HBV infection (398).

Pre-S/S Regions

The pre-S/S region codes for two RNA transcripts that are separately regulated by the pre-S1 promoter and the pre-S2/S promoter. The larger transcript encompassing the pre-S1, pre-S2, and S regions is translated into the large S protein. The smaller transcript encompassing the pre-S2 and S regions is translated into the middle S and small S proteins. The large S protein is essential for virion formation. The middle S protein is dispensable. The small S protein, HBsAg, is the major envelope protein. The "a" determinant of HBsAg is the predominant B cell epitope, and is common to all HBV serotypes. It is a conformational epitope.

S Gene Mutations

Vaccines

Mutations in the *S* gene have been described in infants born to carrier mothers despite adequate anti-HBs response after vaccination (399,400,401). The most common mutation involves a glycine to arginine substitution at codon 145 (G145R) in the "a" determinant (Table 29.9). This mutation has been shown to exhibit decreased binding to monoclonal anti-"a" antibodies thereby allowing the virus to escape neutralization. The G145R mutant has now been reported in many countries. Chimpanzee experiments demonstrated that this mutant is infectious and pathogenic (402). These findings raise concerns about the possibility of widespread dissemination of vaccine escape mutants. A recent study from Taiwan found that the prevalence of HBV S mutants among HBsAg-positive children increased after the introduction of universal vaccination, from 7.8% in 1984 to 23% in 1999 (403,404,405). However, the carrier rate among Taiwanese children decreased from 9.8% to 0.7% (31) during the same period. Another study among 784 preschool children in four Pacific Island countries, who received HBV vaccine failed to detect any "a" determinant mutation (406). These data suggest that the emergence of vaccine escape mutants occurs rarely and has not diminished the efficacy of HBV vaccines. Apart from the G145 R mutant, other mutations in the 'a' determinant have also been reported in lower frequencies (407). Many of these mutants have also been shown to reduce binding to monoclonal anti-"a" antibodies. This raises concerns that some of the S mutants may yield false negative results in serology assays. However, practically all the S mutants that have been identified to date can be detected in HBsAg assays that utilize polyclonal anti-HBs for capture and/or detection.

Liver transplant recipients

Mutations in the HBV *S* gene have also been reported in liver transplant recipients who developed HBV reinfection despite prophylaxis with monoclonal or polyclonal anti-HBs (HBIG) (408,409,410). Most of the mutations are

located in the "a" determinant, the most common being the G145R mutation. One study found a direct correlation between the incidence of the G145R mutant and the duration of HBIG administration, and reversal of the mutation after cessation of HBIG (410). Prophylaxis with combination of HBIG and lamivudine decreases the risk of immune escape and rate of reinfection (411,412,413,414).

Pre-S Mutations

Mutations in the pre-S1 and pre-S2 regions have also been described in patients with chronic HBV infection (415,416,417). The prevalence and the clinical significance of most of these mutations are uncertain. Several investigators reported a high incidence of deletions or mutations in pre-S1 that may cause dysregulation of small S protein synthesis and secretion (418). Retention of HBsAg has been demonstrated to induce liver injury and HCC in transgenic mice (174).

P Gene Mutations

The HBV polymerase gene consists of four distinct regions: A primer involved in priming of reverse transcription, a spacer with no known function, a reverse transcriptase/DNA polymerase which is responsible for reverse transcription of the pregenomic RNA into the first (−) strand HBV DNA and for synthesis of the second (+) strand HBV DNA, and an RNAse H which removes the RNA template (419). The reverse transcriptase/DNA polymerase region has five conserved domains: A, B, C, D, E. Domains A, C and D are involved in nucleoside triphosphate binding and catalysis, whereas domains B and E participate in the positioning of the RNA template and the primer relative to the catalytic site (420,421). The putative catalytic domain is believed to reside in the tyrosine–methionine–methionine–aspartate (YMDD) locus in domain C. This locus is conserved in all viral reverse transcriptases as well as in all isolates of hepadnaviruses (419). Naturally occurring HBV polymerase gene mutations are rarely reported. The most common *P* gene mutations have been found in association with the use of nucleoside/nucleotide analogs for the treatment of chronic HBV infection.

X Gene Mutations

The HBV *X* gene regulates HBV replication through activating and regulating viral and cellular genes. Several studies found that mutations affecting codons 130 and 131 of the *X* gene are more common in patients with HCC (308,390,391) but these *X* gene mutations correspond to the dual core promoter mutations $A_{1762}T$, $G_{1764}A$, which have also been found in many patients with nonmalignant HBV-related chronic liver disease. Therefore, the role of *X* gene mutations in the development of HCC remains to be established.

Treatment

The main goal of treatment of chronic hepatitis B is to prevent cirrhosis, hepatic failure and HCC. This goal is best achieved by eradicating HBV before there is irreversible liver damage. However, it is not possible to eradicate HBV because of the presence of extrahepatic reservoirs of HBV, the integration of HBV DNA into the host genome, and the presence of an intracellular conversion pathway that replenishes the pool of

transcriptional templates (covalently closed circular HBV DNA [cccDNA]) in the hepatocyte nucleus without the need for reinfection (253). Currently, there are five approved treatments for hepatitis B: Standard and pegylated IFN-α, and three nucleoside/nucleotide analogs—lamivudine, adefovir, and entecavir. Many new antiviral and immunomodulatory therapies are being evaluated; some have shown promise and may play a key role in the treatment of chronic HBV infection (Table 29.10).

Definition of Response and Endpoints of Treatment

At the 2000 National Institutes of Health workshop on Management of Hepatitis B, it was proposed that definition and criteria of response to antiviral therapy of chronic hepatitis B be standardized. The proposal categorized responses as biochemical (BR), virological (VR), or histologic (HR), and as on-therapy or sustained off-therapy (Table 29.11) (141). The increased availability of sensitive PCR assays for quantification of HBV DNA has called for more stringent definition of virological response, suppression of serum HBV DNA to

undetectable level by PCR ($<10^2$ IU/mL), particularly for patients with HBeAg-negative chronic hepatitis.

Table 29.10. Treatment of Chronic Hepatitis B

APPROVED TREATMENTS IN THE UNITED STATES
Standard Interferon-α 2b (Intron A)
Pegylated Interferon-α 2a (Pegasys)
Lamivudine (Epivir, 3TC)
Adefovir dipivoxil (Hepsera)
Entecavir (Baraclude)
TREATMENTS APPROVED FOR HUMAN IMMUNODEFICIENCY VIRUS WITH EFFICACY AGAINST HEPATITIS B VIRUS
Emtricitabine (Emtriva, FTC)
Tenofovir (Viread)
Emtricitabine + Tenofovir (Truvada)

Table 29.11. Definition of Response to Antiviral Therapy of Chronic Hepatitis B

CATEGORY OF RESPONSE	
Biochemical (BR)	Decrease in serum ALT to within the normal range
Virological (VR)	Decrease in serum HBV DNA to $<10^5$ IU/mL and loss of HBeAg in patients who were initially HBeAg positive; undetectable serum HBV DNA ($<10^2$ IU/mL) in patients who were initially HBeAg negative
Histologic (HR)	Decrease in histologic activity index by at least two points compared to pretreatment liver biopsy
Complete (CR)	Fulfill criteria of biochemical and virological response and loss of HBsAg
TIME OF ASSESSMENT	
On-therapy	During therapy
Maintained	Persist throughout the course of treatment
End-of-treatment	At the end of a defined course of therapy
Off-therapy	After discontinuation of therapy

Sustained (SR-6)	6 mo after discontinuation of therapy
Sustained (SR-12)	12 mo after discontinuation of therapy

HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.
Adapted from Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000—summary of a workshop. *Gastroenterology* 2001;120(7):1828–1853.

Although IFNs are administered for predefined durations, nucleoside/nucleotide analogs are usually administered until specific endpoint is achieved. The difference in approach is related to the observation that HBeAg seroconversion frequently occurs a few months after cessation of IFN treatment, presumably because of the lag between immune priming and decrease in expression of viral protein; viral rebound is inevitable if nucleoside/nucleotide analogs are withdrawn prior to achieving therapeutic endpoint. For HBeAg-positive patients, virus suppression can be sustained in 50% to 90% patients if treatment is stopped after HBeAg seroconversion is achieved. For HBeAg-negative patients, relapse is frequent even when HBV DNA has been suppressed to undetectable levels by PCR assays for more than a year; therefore, the endpoint for stopping treatment is unclear.

Interferons

IFNs have antiviral, antiproliferative and immunomodulatory effects. IFN-α and -β bind to the same receptor and have predominantly antiviral effects, whereas IFN-γ binds to a separate receptor and has more marked immunoregulatory but less potent antiviral effects. The antiviral effects of IFN depend on its binding to specific receptors, which then triggers a series of intracellular events including activation of 2′ 5′-oligoadenylate synthetase (2′5′-AS). IFNs also have immunomodulatory functions that may be important in eradicating virus infections.

Standard Interferon-α

Efficacy

1. *HBeAg-positive chronic hepatitis*. Clinical studies confirmed that IFN-α suppresses HBV replication. A positive response as defined by loss of viral replication markers (HBeAg and serum HBV DNA by unamplified assays) within 12 months of initiation of treatment can be achieved in 30% to 40% of HBeAg-positive patients who have elevated ALT (Table 29.12) (155,422,423,424,425). Loss of HBsAg is less common and occurs in 5% to 10% of patients only. It should be noted that spontaneous loss of HBeAg can occur in 5% to 15% of untreated controls. Therefore, a treatment-related benefit is only seen in approximately 20% to 25% of patients. Despite the wide range in response rates in reported studies, several meta-analyses confirmed a beneficial effect of IFN therapy (426,427).
    a. *HBeAg-positive patients with normal ALT*. This pattern is usually seen in children or young adults who are in the immune tolerance phase of HBV infection. HBeAg seroconversion occurs in less than 10% of these patients (428,429,430,431).
    b. *Oriental patients*. Asian patients with elevated ALT levels have comparable rates of HBeAg seroconversion as white patients (429). However, a higher proportion of Asian patients have normal ALT levels, so overall response is poor if treatment is extended to include those with normal ALT levels.
    c. *Children*. Ideally, treatment should be instituted as early as possible before there is irreversible liver damage. Among children with elevated ALT, HBeAg clearance has been reported in 30% of those who received IFN-α compared to 10% of controls (432,433,434,435). However, most children, particularly those with perinatally acquired HBV infection have normal ALT and less than 10% of these children who received IFN-α cleared HBeAg (430,431).
2. *HBeAg-negative chronic hepatitis*. Results of four randomized controlled trials involving a total of 86 IFN-α treated patients and 84 controls showed that the end-of-treatment response ranged from 38% to 90% in treated patients compared to 0% to 37% of controls (436,437,438,439). A major problem with

IFN-α treatment of HBeAg-negative chronic hepatitis is the high rate of relapse. Approximately 50% of the responders relapse when therapy is discontinued and relapses can occur up to 5 years post-

therapy. A study from Italy reported a higher rate (30%) of sustained response after 24 months of IFN-α therapy compared to 15% to 20% rates reported in other studies that administered IFN-α for 12 months (438) suggesting that longer duration of treatment may increase the rate of sustained response.

3. *HBV DNA positive clinical cirrhosis*. Patients with histologic cirrhosis but no evidence of hepatic decompensation appeared to tolerate IFN-α treatment and responded as well as patients with precirrhotic chronic hepatitis B (155,429). However, IFN-α even when administered in very low doses (3 MU thrice weekly) is associated with a high risk of serious infections and severe exacerbations leading to hepatic failure in patients with clinically evident cirrhosis (440,441).
4. *Nonresponders to IFN-α treatment*. Most studies found that IFN-α retreatment of HBeAg-positive patients who previously failed to respond to IFN-α was associated with a very low rate of response. One trial of 57 HBeAg-positive patients reported an HBeAg clearance rate of 33% among patients retreated with IFN-α versus 10% in untreated controls (442). However, this trial included patients who were previously treated with suboptimal doses of IFN-α and may have overestimated the benefits of IFN-α retreatment.

Limited data suggest that 20% to 30% HBeAg-negative patients who relapsed or had no response during previous IFN-α treatment had a sustained response after a second course of IFN-α (443).

Role of prednisone priming

Steroid withdrawal has been observed to be frequently accompanied by a flare in serum ALT levels and HBeAg seroconversion. A meta-analysis of seven studies that examined prednisone priming followed by IFN-α treatment (when immune response recovers) found that prednisone priming had very little additional beneficial effect compared to IFN-α alone (444). A subsequent study of 200 European patients reported that patients who received prednisone priming had a significantly higher rate of HBeAg seroconversion compared to those who received IFN-α alone (445). The overall data suggest that a small subset of patients may benefit from prednisone priming but there is a risk of fatal exacerbation in patients with underlying cirrhosis. With the availability of new treatment options, prednisone priming is not recommended for patients with chronic hepatitis B.

Long-term outcome of therapy with interferon-α

Hepatitis B e antigen–positive patients

Most HBeAg-positive patients who responded to IFN-α therapy are able to maintain their response unless they become immunocompromised. Delayed reactivation has been observed in 10% to 20% of responders only, most of which occurred within 1 year of cessation of treatment (154,446). Nevertheless, complete disappearance of markers of HBV replication as determined by PCR assay for serum HBV DNA is seldom achieved in patients who cleared HBeAg only. In addition, several studies reported that the 5-year cumulative rates of HBeAg clearance were similar in treated patients and controls (447,448,449) suggesting that the main role of IFN-α may be to reduce the duration of active liver disease by hastening viral clearance.

Among the sustained responders, an increasing proportion cleared HBsAg during the course of follow-up. However, the percent of sustained responders who cleared HBsAg within 5 years of HBeAg clearance varied from 65% in one US study (446) to 19% to 24% in European studies (447,450,451,452) to 0% to 9% in two studies from Asia (154,453) despite similar durations of follow-up. A sustained antiviral response, especially in those who clear both HBeAg and HBsAg, is almost invariably accompanied by normalization of ALT levels and decrease in necroinflammation but residual liver damage may be present. Data to substantiate the hypothesis that a sustained antiviral response can lead to decreased risks of cirrhosis and HCC and improved survival are limited because chronic hepatitis B is an insidious disease and complications may not be evident until decades later. In addition, patients initially randomized to the control group frequently receive treatment after completion of the trial. There has been only one report comparing the outcome of IFN-α–treated patients and controls. An 8-year follow-up of 101 male patients (67 IFN-α–treated and 37 untreated controls) who participated in a controlled trial of IFN-α therapy in Taiwan found that treated patients had a lower incidence of HCC (1.5% vs. 12%, $P = 0.04$) and a higher survival rate (98% vs. 57%, $P = 0.02$) but there was no difference in the rate of new onset of cirrhosis (453). However, clinical benefits were not observed in a follow-up report from Hong Kong of 208 IFN-α treated and 203 untreated controls (449). IFN-α has not been shown to decrease the incidence of HCC in European or North American patients probably because of the low rate of HCC in untreated patients but studies comparing the outcome of responders vs nonresponders found that patients who cleared HBeAg had better overall survival and survival free of hepatic decompensation; a clinical benefit was most evident among patients with cirrhosis (427,447,451,454,455).

Hepatitis B e antigen–negative patients

Contrary to HBeAg-positive patients, relapse after cessation of IFN-α treatment is frequent, with sustained response rates of only 15% to 30% (443,456). Among the long-term responders, approximately 20% cleared HBsAg after 5 years of follow-up (457). IFN-α therapy did not have any overall effect on survival, complication-free survival or HCC but patients with sustained response had significantly lower rates of hepatic decompensation (456,457).

Dose regimen

IFN-α is administered as subcutaneous injections. The recommended dose for adults is 5 MU daily or 10 MU thrice weekly and for children 6 MU/m^2 thrice weekly with a maximum of 10 MU.

Hepatitis B e antigen–positive patients

The recommended duration of treatment for patients with HBeAg-positive chronic hepatitis B is 16 to 24 weeks. There are very little data to support the use of a longer duration of IFN-α treatment in HBeAg-positive patients. One study reported that among patients who have not cleared HBeAg after 16 weeks of IFN-α, those randomized to continue treatment until week 32 had significantly higher rates of HBeAg clearance compared to those who stopped treatment (458). However, other studies found that response rates after 24 or 12 weeks of IFN-α therapy were similar.

Hepatitis B e antigen–negative patients

Current data suggest that patients with HBeAg-negative chronic hepatitis B should be treated with IFN-α for at least 12 months; one study suggested that higher rates of sustained response can be achieved with 24 months of treatment but that study did not include a comparison group with 12 months treatment (438).

Table 29.12. Comparison of Percentages of Patients with Antiviral Responses to Approved Treatments of Hepatitis B

Response	Standard IFN	Pegylated IFN	Lamivudine	Adefovir	Entecavir	No treatment
HBeAg+ patients	**12–24 wk**	**48 wk**	**52 wk**	**48 wk**	**48 wk**	**48–52 wk**
Undetectable HBV DNA	NA	10–25/7–14	40–44	21	67	0–16
HBeAg seroconversion	~15/~20	22–27/29–32	16–18	12	21	4–10
Loss of HBsAg	1–8	3	<1	0	NA	0–2
Normalization of ALT	~20	34–39/32–41	41–72	48	68	7–24
Histologic improvement	NA	38	49–56	53	72	~25
Durability of response	80–90	~80	50–80	~90[a]	~80	NA
HBeAg– patients	**6–12 mo**	**48 wk**	**52 wk**	**48 wk**	**48 wk**	**48–52 wk**
Undetectable HBV DNA	~60	63/19	50–70	51	90	<10
Normalization of ALT	60–70	38/59	60–70	72	78	~30

Histologic improvement	NA	60	64	70	48	30–40
Durability of response	~20	15	<10	<10	NA	NA

[a]Median duration of treatment = 80 wk.
IFN, interferon; HBV, hepatitis B virus; ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

Pegylated Interferon-α

The attachment of polyethylene glycol to a protein (pegylation) reduces its rate of absorption following subcutaneous injection, reduces renal clearance, and decreases immunogenicity of the protein, with resultant increase in the half-life of the pegylated protein. PegIFN-α has the advantage of more convenient administration and more sustained virus suppression. Clinical trials suggest that efficacy of pegIFN-α is similar or slightly better than standard IFN-α.

Efficacy

1. *HBeAg-positive chronic hepatitis*. In one phase II trial, 194 patients were randomized to receive 90, 180 or 270 μg pegIFN-α2a weekly or 4.5 MU standard IFN-α2a thrice weekly for 24 weeks (Table 29.12). A higher percent of patients who received pegIFN-α had HBeAg seroconversion, 32% versus 25% of those who received standard IFN-α (459). Although this study used a suboptimal dose of standard IFN-α, the convenience of once weekly dosing has led to the replacement of standard IFN with pegIFN. In a subsequent phase III trial, 814 patients were randomized to receive pegIFN-α2a 180 μg weekly, pegIFN-α2a 180 μg weekly + lamivudine 100 mg daily, or lamivudine 100 mg daily for 48 weeks. At the end of treatment, virus suppression was most marked in the group that received combination therapy, mean HBV DNA reduction in the three groups was 4.5, 7.2, and 5.8 $\log_{10}$ copies/mL, respectively (460). HBeAg seroconversion was similar in the three groups at the end of treatment: 27%, 24%, and 20%, respectively, but significantly

higher in the two groups that received pegIFN-α when response was assessed 24 weeks after treatment was stopped: 32%, 27%, and 19%, respectively. These data indicate that pegIFN-α2a monotherapy was superior to lamivudine monotherapy in inducing HBeAg seroconversion, and comparable to the combination therapy of pegIFN-α2a and lamivudine. Similar results were reported in two trials, in which pegIFN-α2b was administered in tapering doses (100 → 50 μg) for 52 weeks or 1.5 μg/kg for 32 weeks. Twenty-four weeks after treatment was stopped, one study reported identical rates (29%) of HBeAg seroconversion in patients who received pegIFN-α2b monotherapy with and without lamivudine (359), whereas the other study reported a significantly higher rate of HBeAg seroconversion in those who received a combination of pegIFN-α2b and lamivudine versus those who received lamivudine only, 36% versus 14% (461). Follow-up of patients in the latter study found that a significant difference in HBeAg seroconversion was maintained up to 76 weeks after treatment was stopped (462).

2. *HBeAg-negative chronic hepatitis*. In the only published report of peg IFN-α in HBeAg-negative patients, 552 patients were randomized to receive 48 weeks of pegIFN-α2a 180 μg weekly, combination of pegIFN-α2a 180 μg weekly + lamivudine 100 mg daily, or lamivudine 100 mg daily. As in HBeAg-positive patients, virus suppression was most marked in the group that received combination therapy, mean HBV DNA reduction at week 48 in the three groups was 4.1, 5.0, and 4.2 $\log_{10}$ copies/mL, respectively (463). However, sustained response (HBV DNA undetectable by PCR and normalization of ALT at week 72) was comparable in the groups that received pegIFN-α2a alone or in combination with lamivudine, and superior to the group that received lamivudine monotherapy: 15%, 16%, and 6%, respectively.

Dose regimen

Currently, only pegIFN-α2a is approved for the treatment of chronic hepatitis B. The recommended dose is 180 μg weekly for 48 weeks. However, given the similarity in response rates between 90 and 180 μg doses in the phase II trial, and the comparable response rates between 24 and 48 week treatment in the phase II and phase III trials (459,460), it is possible that lower doses and/or shorter duration of treatment may suffice for HBeAg-positive patients. Whether longer duration of treatment (>48 week) will result in higher

rates of sustained response in HBeAg-negative patients remains to be determined.

Although the two formulations of pegIFN-α likely have similar efficacy, the optimal dose and duration of pegIFN-α2b for hepatitis B is unclear.

Adverse effects of interferon-α therapy

IFN-α therapy is associated with a broad spectrum of side effects (464). The most common side effect is an initial influenza-like illness: Fever, chills, headache, malaise and myalgia. Other common side effects include fatigue, anorexia, weight loss and mild increase in hair loss. IFN-α has myelosuppressive effects but significant neutropenia (<1,000/mm^3) or thrombocytopenia (<60,000/mm^3) requiring dose reduction or premature termination are uncommon except in patients who have decreased cell counts prior to treatment. The most troublesome side effect of IFN-α is emotional lability: Anxiety, irritability, depression and even suicidal tendency. These symptoms can occur in the absence of a prior history of emotional problems. IFN-α has been reported to induce the development of a variety of autoantibodies. In most instances, this is not accompanied by clinical illness. However, both hyper- and hypo-thyroidism that require treatment have been reported. In addition, there have been reports of worsening liver disease as a result of IFN-α induced exacerbation of an underlying autoimmune hepatitis. Rarely have retinal changes and even impaired vision been reported. Side effects of pegIFN-α are similar to that of standard IFN-α.

Predictors of response to standard and pegylated interferon-α

Predictors of response to standard and pegIFN-α are similar. The strongest predictor of response in HBeAg-positive patients is pretreatment ALT level (429,465). Other factors that have been identified to be associated with a higher rate of IFN-related HBeAg seroconversion include high histologic activity index, low HBV DNA level, and more recently HBV genotypes A and B (359,360,361). There is no consistent predictor of sustained response among HBeAg-negative patients. Some studies found that rapid normalization of ALT and high titer IgM anti-HBc are associated with a higher rate of sustained response but they have not been validated by other studies.

Interferon-β/γ

Early studies using IFN-β from fibroblast cultures were disappointing. More encouraging results were obtained using recombinant IFN-β, but the data are limited. IFN-γ can inhibit HBV replication, but the antiviral efficacy is less than that of IFN-α. In vitro studies suggest that IFN-γ may have anti-fibrotic effects but its in vivo efficacy remains to be proven. Combination therapy of IFN-γ with IFN-α/β has no additional antiviral effects.

Lamivudine (Epivir-Hepatitis B Virus, Triphosphate)

Lamivudine is the (−) enantiomer of 2′,3′-dideoxy-3′-thiacytidine. It is phosphorylated to the triphosphate (3TC-TP) which competes with dCTP for incorporation into growing DNA chains causing chain termination. This may occur during reverse transcription of the first strand as well as during synthesis of the second strand HBV DNA (466).

Efficacy

Lamivudine monotherapy is effective in suppressing HBV replication and in ameliorating liver disease (Table 29.12). One-year treatment with lamivudine results in similar rate of HBeAg seroconversion as 16 week of standard IFN-α, but is inferior to a 1-year course of pegIFN-α. Longer duration of treatment is associated with higher rates of HBeAg seroconversion but also increasing rates of drug-resistant mutations.

1. *HBeAg positive chronic hepatitis B*. Three randomized clinical trials involving a total of 731 patients who received lamivudine for 1 year reported that HBeAg seroconversion occurred in 16% to 18% of treated patients compared to 4% to 6% of untreated controls (467,468,469). Histologic improvement defined as reduction in necroinflammatory score 2 points or greater was observed in 49% to 56% treated patients and in 23% to 25% controls. HBeAg seroconversion rates increased with the duration of treatment to approximately 50% at 5 years (470,471,472,473). Whether the incremental HBeAg seroconversion can be attributed to the additional years of lamivudine treatment is unclear because an untreated control group was not available for comparison.
    a. *HBeAg-positive patients with normal ALT*. As with IFN-α therapy, HBeAg seroconversion rate is less than 10% in patients with pretreatment ALT less than two times normal after 1 year and 19% after 3 years of treatment (156,465,471).
    b. *Oriental patients*. Oriental patients respond similarly to lamivudine as whites (465,467,468,469).
    c. *Children*. Experience with lamivudine in children is limited. One controlled trial involved 286 children, aged 2 to 17 years, with ALT more than 1.3 times normal randomized in a 2:1 ratio to receive lamivudine (3 mg/kg per day up to 100 mg/day) or placebo for 52 weeks. A significantly higher proportion of treated children developed HBeAg seroconversion compared to placebo

controls, 22% versus 13% (474). As with adults, HBeAg seroconversion rate was higher among children with pretreatment ALT greater than three times normal: 34% versus 16% than those with ALT less than or equal to two times normal: 12% versus 8%. Lamivudine was well tolerated but the benefit must be carefully balanced against a high (19%) rate of drug resistant mutations.

2. *HBeAg-negative chronic hepatitis B*. Lamivudine has been shown to benefit patients with HBeAg-negative chronic hepatitis B (475,476,477,478,479). In the only placebo-controlled study, virological and biochemical response (HBV DNA undetectable by bDNA assay and normalization of normal ALT) was achieved in 34 of 54 (63%) patients who received 24 weeks of lamivudine therapy versus 3 of 53 (6%) patients on placebo ($P < 0.001$) (475). Several studies have shown that HBV DNA is suppressed to undetectable levels by PCR assay in 60% to 70% patients after 1 year of treatment. However, the vast majority (approximately 90%) of patients relapsed when treatment was stopped (476). Extending the duration of treatment results in progressively lower rates of maintained response due to selection of drug-resistant mutants. In one study of 78 patients, virologic remission (HBV DNA undetectable by PCR assay) decreased from 77% at 1 year to 52% at 2 years and 42% at 3 years; the corresponding rates of biochemical remission were 90%, 63%, and 53%, respectively (480).
3. *Nonresponders to* IFN-α *treatment*. In a multicenter trial on IFN-α nonresponders, 238 HBeAg-positive patients were randomized to receive lamivudine monotherapy for 52 weeks, combination of 24 weeks lamivudine and 16 weeks of standard IFN-α or no treatment. Patients who received lamivudine monotherapy had the highest HBeAg seroconversion rate, 18% compared to 12% and 13%, respectively in the other groups (481). These data suggest that patients who failed IFN-α treatment have similar response to lamivudine as treatment-naïve patients and retreatment with combination of standard IFN-α and lamivudine did not confer any added benefit compared to retreatment with lamivudine monotherapy.
4. *Advanced liver disease*. Lamivudine has been shown to delay clinical progression in patients with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and HCC. In a double blind, randomized placebo controlled trial, 651 Asian patients who were HBeAg positive or had detectable HBV DNA by branched DNA assay (>700,000 genome equivalents/mL), and bridging fibrosis or cirrhosis on liver biopsy were randomized to receive lamivudine or placebo in a 2:1 ratio. After a median duration of 32 months, a statistically significant difference was observed between the two groups for overall disease progression (increase in Child-Pugh score, hepatic decompensation or HCC): 7.8% versus 17.7%; as

well as HCC development: 3.9% versus 7.4% (332). The benefit was observed mainly among the patients who did not have breakthrough infection. These data indicate that antiviral therapy can improve clinical outcome in patients who have maintained virus suppression. Whether a similar benefit can be observed in patients who have lower serum HBV levels is unclear. It is also uncertain if clinical benefit can be maintained if treatment is stopped after HBV has been suppressed for several years.

5. *HBV DNA–positive clinical cirrhosis*. Lamivudine does not have myelosuppressive effect and rarely induces significant ALT flares during treatment. Therefore, it is a safer treatment than IFN-α in patients with decompensated cirrhosis. In one study of 35 patients (10 with Child's C and 25 with Child's B), improvement in liver disease defined as a decrease in Child-Pugh score of 2 or more was observed in 22 of 23 patients who received a minimum of 6 months treatment (482). However, seven patients had progressive liver disease necessitating liver transplant and an additional five died during the first 6 months. Among the initial responders, two had since died and three had developed breakthrough infection. These data indicate that clinical improvement is slow. The delay in clinical benefit was confirmed in a retrospective analysis of 154 patients who received lamivudine for HBsAg-positive decompensated cirrhosis (483). Of the 32 deaths, 25 (78%) occurred during the first 6 months. Among the patients who survived more than 6 months, the estimated 3-year actuarial survival was 88%. Multivariate analyses showed that high pretreatment bilirubin, creatinine, and HBV DNA levels were significantly associated with 6-month mortality.

Data from these and other studies demonstrate that lamivudine is safe and can stabilize or improve liver function in patients with decompensated cirrhosis thereby obviating or delaying the need for liver transplant (482,483,484,485). However, these studies showed that HCC can still occur even among patients with clinical improvement; therefore, continued surveillance is warranted.

Predictors of response

Hepatitis B e antigen–positive patients

Pretreatment serum ALT is the strongest predictor of response. Multivariate analysis of the data from the multicenter Asian study found that lamivudine treatment and pretreatment serum ALT but not pretreatment serum HBV DNA level or histologic activity index correlated with HBeAg seroconversion (156). Pretreatment serum ALT remained the most important predictor of response when data from four studies with a total of

406 patients who received lamivudine for 1 year were pooled for analysis. HBeAg seroconversion occurred in 2%, 9%, 21%, and 47% patients with pretreatment ALT levels within normal, one to two times normal, two to five times normal and more than five times normal; while the corresponding figures for 196 patients in the placebo group were 0%, 5%, 11%, and 14%, respectively (465).

Hepatitis B e antigen–negative patients

There are no data on predictors of response to lamivudine treatment of HBeAg-negative patients.

Durability of response

Durability of HBeAg seroconversion has been reported to vary from 50% to 80%. In a follow-up report of patients in the United States and Europe, who completed 1 year of lamivudine treatment, 30 of 39 (77%) patients with HBeAg seroconversion had a durable response after a median follow-up of 37 months (range 5 to 46 months) (486). In addition, 8 (20%) patients developed HBsAg seroconversion. The estimated durability of lamivudine-induced HBeAg seroconversion was lower, 64% at 36 months, based on intention to treat analysis. Studies from Asia reported lower rates of durability—50% to 60% (365,487,488,489,490), in part related to a shorter duration of treatment, mean 8 to 9 months. Several factors have been identified to be associated with increased durability of HBeAg seroconversion including longer duration of consolidation treatment (continued treatment after HBeAg seroconversion), younger age, lower HBV DNA level at the time treatment was stopped, and genotype B versus C, the most consistent factor appears to be duration of consolidation treatment (365,488,489,490). Although there are no good direct comparison data, it appears that durability of lamivudine-induced HBeAg seroconversion is less than that for IFN (491).

Among HBeAg-negative patients, durability of virus suppression after 1-year of lamivudine treatment is less than 10%. One study reported that durability of virologic response (undetectable HBV DNA by PCR assay) can be improved to 50% in patients who have completed 2 years treatment and had undetectable HBV DNA by PCR assay on at least three consecutive occasions (3 months apart) (492). Confirmation of these data may help in identifying a subset of patients who do not need to be on life-long treatment.

Lamivudine resistance

Selection of lamivudine resistant mutants is the main concern with lamivudine treatment. The most common mutation affects the YMDD motif of the HBV DNA

polymerase (M204V/I). This mutation is frequently accompanied by L180M (493,494), other mutations that have been commonly observed include V173L and changes at L80. Lamivudine resistance is usually manifested as breakthrough infection defined as greater than 1 $\log_{10}$ increase in serum HBV DNA from nadir. However, breakthrough infection also can be a result of noncompliance.

Genotypic resistance can be detected in 14% to 32% of HBeAg-positive patients after 1 year of lamivudine treatment (467,468,469) and increases with the duration of treatment to 60% to 70% after 5 years of treatment (470,472,473). Retrospective analysis of 998 HBeAg-positive patients who received a median of 4 years of lamivudine treatment identified Asian ethnicity, high pretreatment serum HBV DNA levels, male gender, and longer duration of lamivudine treatment as factors that correlated with the development of lamivudine resistance (473). One study found that a high level of residual virus: Serum HBV DNA greater than 10^3 copies/mL after 6 months of treatment was associated with a higher rate of lamivudine resistance: 63% versus 13% (495). The rates of lamivudine resistance in patients treated for HBeAg-negative chronic hepatitis B appear to be more variable, 0% to 27% at 1 year and 10% to 56% at 2 years (475,476,477,496).

The clinical course of patients with lamivudine resistant mutants is variable. In vitro studies showed that M204V/I mutation decreases replication fitness of HBV (497,498) but compensatory mutations selected during continued treatment, such as L180M, can restore replication fitness. Therefore, serum HBV DNA levels tend to be lower than baseline when breakthrough infection is first diagnosed. However, over time, serum HBV DNA levels increase and may become higher than pretreatment values. Virologic breakthrough is usually followed by biochemical breakthrough (480). In some patients emergence of lamivudine resistant mutants may be accompanied by acute exacerbations of liver disease (499,500) but exacerbations associated with emergence of lamivudine resistance may also lead to HBeAg seroconversion (470,501). The frequency of hepatitis flares increases with the duration of lamivudine resistance, from 43% in year 1 to greater than 80% after 3 years (473). The occurrence of icteric flares and hepatic decompensation is rare in young precirrhotic patients (6% after 4 years of lamivudine resistance), but more common and may be fatal in older patients and those with advanced fibrosis or cirrhosis. Withdrawal of treatment in patients who have developed lamivudine resistance has been reported to be associated with rapid outgrowth of wild-type virus and flares in liver disease (502,503). However, two studies in Asia found that hepatitis flares and decompensation were similar among patients with lamivudine breakthrough, who stopped or continued lamivudine treatment (504,505).

Long-term outcome of lamivudine-treated patients

Follow-up of patients receiving continued lamivudine treatment showed that the rates of maintained virologic and biochemical response decrease with time due to selection of drug-resistant mutants. As a group, liver histology after 3 years of treatment is improved compared to baseline but histologic benefit after the first year of treatment is negated among patients with breakthrough infection (479,486). Despite increasing rates of breakthrough infection, two studies with median follow-up of 2 to 4 years reported that lamivudine treatment decreased the overall rate of hepatic decompensation as well as liver-related mortality (506,507).

Adverse events

In general, lamivudine is very well tolerated. Various adverse events including a mild (two- to threefold) increase in ALT level have been reported in patients receiving lamivudine, but these events occurred with the same frequency among the controls.

Dose regimen

The recommended dose for adults with normal renal function (creatinine clearance >50 mL/minute) and no HIV infection is 100 mg daily PO. Dose reduction is necessary for patients with renal insufficiency. Patients with HIV coinfection should be treated with 150 mg b.i.d. doses in addition to other antiretroviral therapies.

Hepatitis B e antigen–positive patients

The end point of treatment for HBeAg-positive patients is HBeAg seroconversion. In general, lamivudine should be administered for a minimum of 6 months after confirmed HBeAg seroconversion. With the availability of newer treatments with lower risk of drug resistance, whether lamivudine should be continued in patients who have been on treatment for more than a year and have not achieved HBeAg seroconversion nor developed breakthrough infection or switched to new therapies is unclear. For patients who have breakthrough infection due to drug resistance, the vast majority should receive rescue therapy with antiviral agents that are effective against lamivudine-resistant HBV mutants. A minority of patients who do not have underlying cirrhosis or immunosuppression may consider stopping treatment, particularly if the indications for initial treatment were weak.

Acute exacerbations of hepatitis with or without hepatic decompensation may occur after discontinuation of lamivudine therapy. Exacerbations may occur even in patients who have developed HBeAg seroconversion and may be up to 1 year (median 4 months) after

cessation of treatment (501,502). Therefore, all patients should be closely monitored after treatment is discontinued. Reinstitution of lamivudine treatment is usually effective in controlling the exacerbations in patients who have not developed breakthrough infection, and may result in subsequent HBeAg seroconversion but the benefits of retreatment are usually transient in patients with breakthrough infection as resistant mutants are quickly selected when lamivudine treatment is resumed (496,503).

Hepatitis B e antigen–negative patients

The end-point of treatment for HBeAg-negative chronic hepatitis B is unknown. Post-treatment relapse can occur even in patients with undetectable serum HBV DNA by PCR assay. Because of the high rate (approximately 90%) of relapse in patients who responded after 1 year of treatment, longer duration of treatment is recommended. However, the criteria for discontinuation of treatment and the optimal duration of therapy have not been established.

Famciclovir

Famciclovir is the oral prodrug of penciclovir, an acyclic deoxyguanosine analog. Penciclovir is phosphorylated to its triphosphate (PCV-TP) which competes with 2′-deoxyguanosine-5′-triphosphate (dGTP) for incorporation into the nascent HBV DNA chains. In addition, PCV-TP may compete with dGTP for the priming of reverse transcription (synthesis of the first DNA strand) (508).

A phase III clinical trial of 417 patients with HBeAg-positive chronic hepatitis B found that the median decrease in histologic activity index among the patients who received famciclovir 500 mg t.i.d., famciclovir 1,500 mg daily or placebo were 1.5, 1, and 0, respectively. Compared to controls, a higher rate of HBeAg seroconversion was observed among the patients who received famciclovir 500 mg t.i.d. (9% vs. 3%), but not in the group who received famciclovir 1,500 mg daily (509).

Drug resistant mutants have also been reported in patients who have been on long-term famciclovir treatment (510). In view of the low efficacy, need for administration thrice daily, and potential for cross-resistance with lamivudine, it is unlikely that famciclovir will have a major role in the treatment of chronic hepatitis B.

Adefovir Dipivoxil (Hepsera, Bis-Pom PMEA)

Adefovir is a nucleotide analog of adenosine monophosphate. It can inhibit reverse transcriptase as well as

DNA polymerase activity (511). In vitro and clinical studies showed that it is effective in suppressing wild type as well as lamivudine-resistant HBV (512).

Efficacy

1. *HBeAg-positive chronic hepatitis*. A phase III clinical trial included 515 patients with compensated liver disease randomized to receive two doses of adefovir (30 or 10 mg daily) or placebo (Table 29.12) (513). After 48 weeks of treatment, histologic improvement was observed in 59%, 53%, and 25%, respectively. Median decrease in serum HBV DNA level was -4.8, -3.5, and -0.6 $\log_{10}$ copies/mL, while the proportion of patients with undetectable HBV DNA by PCR assay was 39%, 21%, and 0, respectively. Normalization of ALT was observed in 55%, 48%, and 16% whereas HBeAg seroconversion occurred in 14%, 12%, and 6%, respectively. All assessments of response showed a statistical difference between the two treatment groups and the placebo group, and a trend indicating superiority of the higher dose (30 mg) group. However, the 30-mg dose was associated with nephrotoxicity albeit less frequently than the doses (60 and 120 mg) initially used for HIV infection.
2. *HBeAg-negative chronic hepatitis*. A phase III clinical trial included 185 patients with compensated liver disease randomized to receive adefovir 10 mg daily or placebo (514). After 48 weeks of treatment, patients who received adefovir were more likely to have improvement in liver histology (77% vs. 33%), undetectable HBV DNA by PCR assay (51% vs. 0), and normalization of ALT (72% vs. 29%). A follow-up report of 70 patients found that after 144 weeks of continued adefovir treatment, median HBV DNA decrease compared to baseline was 3.6 $\log_{10}$ copies/mL, 79% patients had undetectable HBV DNA by PCR, and 69% patients had normalization of ALT (515). Two patients lost HBsAg. These data indicate that response can be maintained in 70% to 80% patients but as many as 20% to 30% failed to achieve virologic or biochemical response after 3 years of continued adefovir treatment.
3. *Decompensated cirrhosis/liver transplantation*. Data on adefovir as de novo therapy in patients with decompensated cirrhosis or liver transplantation are not available. It is likely that the efficacy will be similar to that in patients who received adefovir as salvage therapy for lamivudine-resistant HBV.
4. *Lamivudine-resistant HBV*. Clinical trials confirmed that adefovir is effective in suppressing lamivudine-resistant HBV (516,517,518,519). One study that included 19 patients randomized to receive adefovir monotherapy found that antiviral efficacy of adefovir alone was comparable to that of combination therapy of adefovir and lamivudine (516). However, hepatitis

flares were more common during the transition period. In addition, recent data showed that sequential monotherapy increases the risk of adefovir resistance (520). In patients with decompensated liver disease, addition of adefovir has been shown to result in clinical improvement, reduction in risk of recurrent hepatitis B post–liver transplant, and possibly increased survival (518,519). One study included 128 pre- and 196 post-transplant patients (518). Among the patients who received 48 weeks treatment, 81% of the pre- and 34% of the post-transplant patients had undetectable HBV DNA by PCR assay, and 76% and 49%, respectively had normalization of ALT. Child-Turcotte-Pugh score improved in more than 90% of patients in both groups, and 1-year survival was 84% for the pre- and 93% for the post-transplant patients. Adefovir when added to existing HIV treatment regimen which included lamivudine 150 mg b.i.d. has also been shown to be effective in decreasing serum HBV DNA levels in patients with HIV and HBV coinfection and lamivudine-resistant HBV (521).

Predictors of response

Detailed analyses of predictors of response had not been performed. Retrospective analyses of data from the two phase III clinical trials showed that HBV DNA reduction was comparable across the four major HBV genotypes (A to D) (362), but an association between adefovir-related HBeAg seroconversion or durability of response and HBV genotypes could not be analyzed because of the small number of responders. HBV DNA reduction was also similar among Asians and Caucasians. Limited data suggest that HBeAg-positive patients with high pretreatment ALT were more likely to undergo HBeAg seroconversion and to have histologic improvement.

Durability of response and long-term outcome of adefovir-treated patients

Hepatitis B e antigen–positive chronic hepatitis

Analyses of long-term data were hampered by errors in treatment assignment during the second year of the phase III trial. HBeAg seroconversion rates increased during the second and third years of treatment but the exact figures were unclear. Durability of HBeAg seroconversion was examined in 76 patients who had achieved HBeAg seroconversion after a median of 80
(30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,6

weeks adefovir treatment, and had been followed for a median of 52 (5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,4 weeks off treatment. HBeAg seroconversion was maintained in 69 (92%) patients (522). The seemingly higher rate of durability of adefovir-related HBeAg seroconversion compared to lamivudine may be related to a longer duration of treatment (80 vs. <52 weeks) and more importantly, a longer duration of consolidation treatment.

Hepatitis B e antigen–negative chronic hepatitis

A follow-up report found that almost all 40 patients who received adefovir in year 1 of the phase III trial, who were randomized to placebo in year 2 relapsed. At week 96 (48 weeks after stopping adefovir), only 8% patients had undetectable HBV DNA by PCR assay and 32% had normal ALT (515). These data indicate that longer duration (>48 weeks) of treatment is necessary to achieve sustained response but the optimal duration remains to be determined. Most patients maintained their response when adefovir was continued for 3 years, but there was minimal incremental response during years 2 and 3.

Hepatitis B s antigen loss

A retrospective analysis of 578 patients who received adefovir monotherapy found that nine (1.6%) patients had HBsAg seroconversion after a median of 73 weeks (523), indicating that HBsAg loss is a rare event.

Dose regimen

The approved dose of adefovir is 10 mg daily PO. Although higher doses appeared to have more potent antiviral effect, concerns for nephrotoxicity limit their use in clinical practice. Adefovir at the approved dose of 10 mg daily is ineffective in inhibiting HIV replication.

For HBeAg-positive patients, treatment should be administered for an additional 6 months after HBeAg seroconversion is achieved. For HBeAg-negative patients, long-term treatment is needed but the optimal duration of treatment has not been determined. Patients must be closely monitored for relapse when treatment is stopped. Because of the weak antiviral effects of the 10-mg dose, 25% to 50% patients will have a suboptimal virologic response with less than 3.5 $\log_{10}$ reduction in HBV DNA after 48 weeks of treatment (524); whether these patients should be switched to other more potent antiviral agents such as entecavir and when the switch should occur have not been examined.

Adverse events

Adefovir is in general well tolerated with similar side effect profile as placebo in the phase III clinical trials in patients with compensated liver disease. The most worrisome adverse event is nephrotoxicity—increase in serum creatinine and/or renal tubular defects manifested as hypophosphatemia and Fanconi's syndrome, which was common at high doses (60 to 120 mg daily). A reproducible increase in serum creatinine by greater than or equal to 0.5 mg/dL has not been reported in

patients who received 10 mg doses after 48 weeks and in 6% after 192 weeks of treatment (513,514,515,525). In all cases, the increase in serum creatinine was reversible but a few patients had to be withdrawn from treatment. Nephrotoxicity was more common in patients with decompensated cirrhosis and in those who had undergone liver transplantation; a reproducible increase in serum creatinine by greater than or equal to 0.5 mg/dL was observed in 15% to 22% of these patients after 48 weeks of 10 mg doses (518). Whether the high frequency of nephrotoxicity in this setting is a direct result of adefovir, concomitant nephrotoxic medications, preexisting renal dysfunction or hepatorenal syndrome or a combination of these factors is unclear. Renal function should be monitored in all patients receiving adefovir and dosing intervals adjusted in patients with estimated creatinine clearance less than 50 mL/minute.

Adefovir resistance

Resistance occurs at a slower rate during adefovir treatment compared to lamivudine. Several mechanisms have been suggested that might contribute to the low rate of resistance to adefovir. The adefovir molecule is structurally similar to 2′-deoxyadenosine 5′-triphosphate (dATP), which limits steric discrimination by HBV polymerase. The phosphonate bond in adefovir may be less susceptible to chain terminator removal once the molecule is incorporated into viral DNA. In addition, its flexible molecular structure permits it to bind HBV polymerase even in the presence of minor alterations in the nucleotide binding pocket. Resistance to adefovir has not been detected in clinical trials of patients who received 48 weeks of treatment (526). However, novel mutations conferring resistance to adefovir (asparagine to threonine substitution N236T and alanine to valine or threonine substitution A181V/T) have been described (527,528). Aggregate data from five studies including three studies using combination of adefovir and lamivudine in patients with lamivudine-resistant HBV estimated the cumulative rate of resistance to be 15% by 192 weeks (529). Another study of adefovir monotherapy in 67 HBeAg-negative patients found that the cumulative rate of resistance was 0%, 3%, 11%, and 18% at week 48, 96, 144, and 192 respectively (525).

In vitro studies showed that adefovir-resistant mutations decrease susceptibility by 3 to 15-fold (528,530). Nevertheless, clinical studies found that viral rebound, hepatitis flares and even hepatic decompensation can occur (531). Therefore, all patients receiving long-term adefovir should be closely monitored for resistance. Risk factors for adefovir resistance that have been identified include suboptimal virus suppression and sequential monotherapy. A pooled study of 467 patients with lamivudine-resistant HBV found that resistance to adefovir occurred only in patients who stopped lamivudine (520). Sequential treatment with lamivudine followed by adefovir had also been reported to select dual-resistant HBV mutants.

In vitro and human studies showed that adefovir-resistant HBV mutants are susceptible to lamivudine and entecavir (527,528,531). However, the duration of benefit is unknown, and may be short lived in patients with earlier lamivudine resistance.

Entecavir (Baraclude)

Entecavir is an orally administered cyclopentyl guanosine analog. In vitro studies as well as studies in woodchucks showed that it has potent antiviral effects against HBV. One study found that long-term treatment of woodchucks chronically infected with WHV infection resulted in decreased incidence of HCC and increased survival (334). In vitro studies showed that entecavir is effective in suppressing lamivudine-resistant HBV but susceptibility is reduced compared to wild type HBV (414). Entecavir has also been found to be effective in suppressing adefovir-resistant HBV in in vitro studies. Clinical trials confirmed the efficacy of entecavir in humans (532).

Efficacy

1. HBeAg-positive patients. In a phase III clinical trial 715 patients with compensated liver disease were randomized to receive entecavir 0.5 mg or lamivudine 100 mg daily (Table 29.12). At week 48, entecavir resulted in statistically higher rate of histologic (72% vs. 62%), virologic (HBV DNA undetectable by PCR) (67% vs. 36%) and biochemical (68% vs. 60%) responses (533) compared to lamivudine. However, despite more potent viral suppression (6.9 vs. 5.4 log10 copies/mL), HBeAg seroconversion rates were similar in the two groups: 21% versus 18%.
2. HBeAg-negative patients. In a phase III clinical trial 648 patients with compensated liver disease were randomized to receive entecavir 0.5 mg or lamivudine 100 mg daily. At week 48, entecavir resulted in statistically higher rate of histologic (70% vs. 61%), virologic (90% vs. 72%) and biochemical (78% vs. 71%) responses compared to lamivudine (534).
3. Lamivudine-refractory HBV. In one study 286 patients, who had persistent viremia while on lamivudine with or without confirmed lamivudine-resistant mutations, were randomized to receive entecavir 1.0 g or lamivudine 100 mg daily. At week 48, entecavir resulted in statistically higher rate of histologic (55% vs. 28%), virologic (21% vs. 1%) and biochemical (75% vs. 23%) responses compared to lamivudine (535).

Predictors of response

Predictors of response have not been examined in detail. Entecavir appears to be equally effective in decreasing serum HBV DNA level and in inducing histologic improvement in Asians and Whites, across HBV genotypes A-D and a wide range of pretreatment HBV DNA and ALT levels. However, HBeAg seroconversion rates are lower in patients with pretreatment ALT less than 2 × normal.

Durability of response

Limited data suggest that durability of response to entecavir is similar or better than lamivudine.

Dose regimen

The approved dose for nucleoside-naïve patients is 0.5 mg daily PO and for lamivudine-refractory patients is 1.0 mg daily PO. Doses should be adjusted for patients with estimated creatinine clearance less than 50 mL/minute.

Adverse events

Entecavir had a similar safety profile including on-treatment ALT flares as lamivudine in clinical trials. Studies in mice found an increased risk of lung adenomas (at exposures 3 to 40 times those in humans) (532). In addition, HCC were increased in male mice while brain gliomas were increased in male and female rats. Whether these observations have relevance to humans is unclear. To date, no difference in incidence of HCC or other neoplasms has been observed between patients who received entecavir versus lamivudine but the duration of follow-up is limited.

Entecavir resistance

No resistance was observed after 48 weeks of treatment in the two phase III clinical trials of nucleoside-naïve patients, although resistance was detected in 7% of patients by week 48 in the trial of lamivudine refractory patients (533,534,535). Mutations associated with entecavir resistance have been localized to rtI169, rtT184, rtS202, and rtM250 (536). These mutations on their own have minimal effect on susceptibility to entecavir, but when present with lamivudine-resistant mutations, decrease susceptibility to entecavir by greater than 1,000-fold (537).

Emtricitabine (Emtriva, FTC)

Emtricitabine is a potent inhibitor of HIV and HBV replication. FTC has been approved for HIV treatment as Emtriva (FTC only) and as Truvada (in combination with tenofovir as a single pill). Because of its structural similarity with lamivudine (3TC), treatment with FTC selects for the same resistant mutants.

A phase II trial that included 98 patients (77 HBeAg positive) with chronic hepatitis B randomized to receive varying doses of FTC found that 200 mg had the maximum effect on viral suppression (538).

In another study, 248 patients (63% were HBeAg positive) were randomized to receive FTC 200 mg daily or placebo in a 2:1 ratio (539). At week 48, FTC resulted in a significantly higher rate of histologic (62% vs. 25%), virologic (undetectable HBV DNA by PCR assay) (54% vs. 2%) and biochemical (65% vs. 25%) responses but HBeAg seroconversion rates were identical—12% in the two groups. FTC-resistant mutations were detected in 13% patients who received FTC. The high rate of drug resistance and the lack of improvement on HBeAg seroconversion indicate that FTC on its own has no role in the rapidly expanding treatment armamentarium for hepatitis B.

Tenofovir (Viread)

Tenofovir disoproxil fumarate is a nucleotide analog that has been approved for the treatment of HIV infection as Viread (tenofovir only) or Truvada (tenofovir + emtricitabine as a single pill). Tenofovir is structurally similar to adefovir. In vitro studies showed that tenofovir and adefovir are equipotent. Because tenofovir appears to be less nephrotoxic, the approved dose is much higher than that of adefovir, 300 mg versus 10 mg daily. This may explain why tenofovir has more potent antiviral effects in clinical studies. Tenofovir has not been approved for treatment of hepatitis B; clinical studies designed to evaluate its safety and efficacy in patients with chronic hepatitis B, particularly those with HBV monoinfection and no earlier lamivudine treatment are under way. Currently, most of the data on tenofovir are based on studies of patients with HBV and HIV coinfection, who have lamivudine-resistant HBV.

Retrospective analysis of two large multicenter HIV trials that included subsets of patients with chronic hepatitis B (n = 23) demonstrated that tenofovir was associated with a significant reduction in HBV DNA levels both in patients who had not previously received anti-HIV therapy, as well as those who had already been exposed to anti-HIV therapy (540). Several pilot studies and case series confirmed that tenofovir is effective in decreasing serum HBV DNA levels in patients (with and without HIV coinfection) with lamivudine-resistant HBV (541,542,543,544,545).

In a study of 53 patients with lamivudine-resistant HBV, tenofovir led to a greater reduction in serum HBV DNA levels than adefovir (546). Although the study was not randomized, and there was heterogeneity in patient characteristics and treatment regimen (some but not all continued lamivudine), the finding that

tenofovir is superior to adefovir in clinical practice is not surprising due to the 30-fold difference in dose. Viral rebound has also been reported when patients were switched from tenofovir to adefovir (547).

Tenofovir is less nephrotoxic than adefovir. However, tenofovir has been occasionally reported to cause Fanconi's syndrome and renal insufficiency (548,549,550). Almost all of these adverse events occurred in patients with HIV coinfection receiving antiretroviral therapy. The likelihood of tenofovir-associated nephrotoxicity in patients with HBV monoinfection is unclear. Nevertheless, all patients receiving long-term tenofovir should be closely monitored, particularly those with baseline impaired renal function and those with decompensated liver disease.

L-Deoxythymidine (LdT, Telbivudine) and VAL-Deoxycytosine (VAL-LdC)

L-deoxythymidine (LdT) and val-deoxycytosine (val-LdC) are nucleoside analogs with potent antiviral effects against HBV. However, they select for the same mutations as lamivudine, FTC and clevudine.

A phase II trial included 104 HBeAg-positive patients who were randomized to receive LdT (400 or 600 mg daily) alone or in combination with lamivudine 100 mg daily, or lamivudine alone (551). The two LdT monotherapy groups had higher rates of virologic (61% vs. 32%) and biochemical (86% vs. 63%) responses compared to the lamivudine group. HBeAg seroconversion rates were similar in the two groups: 31% versus 22%. Combination of LdT and lamivudine did not confer any benefit; in fact the combination group appeared to fare worse than the LdT monotherapy group. Mutations in the YMDD motif were detected in 4.5%, 9.8% and 15.8% of patients who received LdT alone, LdT and lamivudine, and lamivudine alone,

respectively. A follow-up report on 90 patients who continued treatment for 96 weeks showed that LdT continued to be superior to lamivudine in virologic and biochemical responses but the difference in HBeAg seroconversion rates was not statistically significant (38% vs. 21%) (552). Mutations in the YMDD motif were detected in six additional patients in the LdT alone group during the second year of therapy, resulting in a cumulative resistance rate of 18% at the end of year 2. Phase III clinical trial is ongoing. Given the availability of newer antiviral agents with lower risk of drug resistance, the role of LdT in hepatitis B treatment is limited.

Val-LdC is a well absorbed oral prodrug of LdC. Phase I/II clinical trials showed that it is effective in suppressing HBV replication (553). In vitro and woodchuck studies found that val-LdC and LdT have additive antiviral effects.

Clevudine (LFMAU, 2′-Fluoro-5-Methyl-L-Arabinofuranosyl Uracil)

Clevudine is a pyrimidine nucleoside analog that is effective in inhibiting HBV replication in vitro and in animal models. Phase I/II clinical trials confirmed that a 4-week course of clevudine can decrease serum HBV DNA levels by 2 to 3 $\log_{10}$ copies/mL and at high doses, HBV DNA levels remained suppressed for up to 24 weeks after stopping treatment (554,555). Clinical trials are ongoing to determine the optimal dose regimen of clevudine and to determine if sustained response can be achieved after a longer (24 week) course of treatment. One drawback of clevudine is that it selects for the same mutations as lamivudine and FTC.

Combination Therapies

Monotherapy with a single antiviral agent or IFN-α is unlikely to be sufficient for the eradication of HBV infection in most patients with chronic hepatitis B. Combination therapies have been proven to be more effective than monotherapy in the treatment of HIV and HCV infection. The potential advantages of combination therapies are additive or synergistic antiviral effects, and diminished or delayed resistance. The potential disadvantages of combination therapies are added costs, increased toxicity, and drug interactions. Various combination therapies have been evaluated; to date, none of the combination therapies has been proven to be superior to monotherapy in inducing a higher rate of sustained response or in decreasing the rate of drug resistance. Nonetheless, continued effort should be expended to develop an optimal combination therapy for hepatitis B.

Standard or pegylated interferon-α and lamivudine

The combination of IFN-α and lamivudine seems logical because monotherapy with each agent is effective, and IFN-α and lamivudine have different mechanisms of action.

Treatment-naïve patients

Five large trials (one using standard IFN-α and four using pegIFN-α, 4 in HBeAg-positive patients and one in HBeAg-negative patients) were conducted comparing combination of IFN-α and lamivudine to lamivudine alone and/or IFN-α alone (359,460,461,463,469). All studies found that combination therapy had greater on-treatment virus suppression but there was no difference in sustained off-treatment virologic response compared to IFN-α alone. All studies showed that

combination therapy resulted in higher rates of sustained off-treatment response compared to lamivudine alone. Although combination therapy was associated with lower rates of lamivudine resistance compared to lamivudine monotherapy, a low rate of lamivudine resistance was encountered compared to none in patients who received IFN-α alone.

Interferon-α nonresponders

Combination therapy of standard IFN-α and lamivudine is not more effective than lamivudine alone in the retreatment of IFN-α nonresponders (481).

Lamivudine and adefovir

Nucleosid(t)e naïve patients

One trial included 115 patients randomized to receive a combination of lamivudine and adefovir or lamivudine alone. At week 52, there was no difference in HBV DNA suppression, ALT normalization or HBeAg loss (556). However, combination therapy was associated with a lower rate of detection of lamivudine-resistant mutations: 20% versus 2% suggesting that the benefit of combination therapy may be observed with continued treatment.

Patients with lamivudine-resistant hepatitis B virus

One small trial in patients with compensated liver disease showed that combination of adefovir and lamivudine was not superior to adefovir alone in decreasing serum HBV DNA levels (516). However, hepatitis flares were less frequent during the transition period in the combination therapy group. Recent

data suggest that continuation of lamivudine reduces the risk of resistance to adefovir (520). There is therefore increasing evidence to support the conclusion that combination of adefovir and lamivudine is superior to adefovir monotherapy for patients with lamivudine-resistant HBV.

Lamivudine and telbivudine

One trial conducted in nucleoside naïve HBeAg-positive patients demonstrated that combination of lamivudine and telbivudine had no advantage over telbivudine alone (551). In fact, the combination group showed a trend toward an inferior result in all parameters: Virus suppression, ALT normalization, HBeAg seroconversion, and mutations in the YMDD motif. These data suggest that lamivudine and telbivudine, both being L-nucleosides, may antagonize each other by competing for the same binding site on the HBV reverse transcriptase.

Adefovir and Emtricitabine

A pilot trial on 30 HBeAg-positive patients found that patients randomized to receive combination of adefovir and emtricitabine had greater decrease in serum HBV DNA levels compared to those who received adefovir alone (557). Whether this was related to an additive or synergistic effect of these two compounds or an unusually poor response in the adefovir monotherapy group (2 to 3 $\log_{10}$ reduction in serum HBV DNA after 1 year of therapy) is unclear.

Novel Antiviral Approaches

Several innovative antiviral approaches have been evaluated in in vitro as well as in animal models of chronic hepatitis.

Selective targeting of antiviral agents to the liver

Conjugation of antiviral agents to ligands that are selectively taken up by the liver may permit these agents to be used in lower doses with decreased systemic adverse effects. Several systems of selective delivery have been evaluated including conjugation to lactosaminated human serum albumin, liposome encapsulation, and incorporation into recombinant chylomicrons (558,559).

Antisense approaches

Transcription and translation of HBV DNA and HBV RNA can be prevented by antisense molecules or ribozymes that are complementary to the DNA or RNA templates (560,561). These molecules can be delivered by the administration of preformed molecules or vector DNA. The advantage of this approach is that specific targets can be precisely selected. In addition, the risk of drug-resistant mutants can be reduced by targeting multiple sites in the viral DNA or RNA or by targeting regulatory sequences that would not tolerate mutations. In vitro studies have confirmed that this approach is feasible. The major impediments to the clinical use of antisense treatment include rapid degradation of the antisense molecules by nucleases in vivo, lack of an efficient delivery system into the target cells, and hindrance of access to target DNA or RNA sequences by secondary structure.

Short interfering RNA

RNA interference (RNAi) is a recently discovered cellular mechanism that detects and destroys double-stranded RNA and seems to play a role in the cell's antiviral defense system (562). Short interfering RNA (siRNA) molecules are approximately 21-nucleotide,

double-stranded RNA intermediates of the RNAi mechanism that guides a unique RNA-induced silencing complex to target RNA, leading to its subsequent degradation. Administration of synthetic siRNAs that use the endogenous cellular mechanism to downregulate the expression of HBV genes is a potential novel approach to treatment of hepatitis B. Several investigators have demonstrated the efficacy of siRNA in inhibiting HBV expression in cell culture systems and more recently in animal models (563,564,565,566,567). Nevertheless, major hurdles in delivering adequate amounts of siRNA to the target cells need to be overcome before this approach can be tested in humans.

Immunomodulatory Therapy

Nonspecific immunomodulation is largely ineffective in clearing HBV infection.

Thymosin

Thymic-derived peptides can stimulate T cell function. Thymosin is well tolerated but data on efficacy are conflicting (568,569,570,571). A meta-analysis that included five controlled trials with a total of 353 patients concluded that patients treated with thymosin were significantly more likely than controls to have a virologic response (572). The maximal rate of response was not seen until 12 months after discontinuing therapy (odds ratio 1.67, 95% CI, 0.83 to 3.37). Thymosin is approved for the treatment of hepatitis B in some countries, mainly in Asia.

Hepatitis B virus–specific immunomodulation

In the past few years, several HBV-specific immunomodulatory therapies have been developed, some of which have shown promise.

S and pre-S antigen vaccines

Several uncontrolled trials reported that vaccines with HBV S with or without pre-S antigens used for prevention of HBV infection were effective in inducing anti-HBs response and in decreasing serum HBV DNA levels in patients with chronic hepatitis B (573,574). These data need to be confirmed in controlled clinical trials.

Deoxyribonucleic acid vaccination

Unlike peptide vaccines, vaccination with plasmid DNA that express viral proteins in situ can stimulate not only B cell but also T cell (both helper and cytotoxic) response. In addition, DNA vaccines lead to more prolonged expression of viral proteins (575). Studies in mice have shown that vaccination with plasmids that contain HBV surface gene can induce anti-HBs response. DNA vaccination has been demonstrated to decrease the production of HBsAg in transgenic mice that express the HBV surface gene (576). One pilot study reported that DNA vaccination was also effective in activating T cell response and in decreasing serum HBV DNA levels in patients with chronic HBV infection (577).

T cell vaccines

Patients with chronic HBV infection have been demonstrated to have impaired cytotoxic T lymphocyte response to HBV antigens, resulting in ineffective virus clearance. In vitro and animal (transgenic mice) studies show that CTL response to HBcAg can be induced by repeated exposure to peptides that correspond to major HBcAg epitopes. Moreover, administration of HBsAg primed CTLs to transgenic mice that express HBV surface gene can result in decreased transcription of viral RNA and translation of viral antigens with minimal cell damage (578). These data suggest that vaccination with synthetic peptides that stimulate CTL response to HBV antigens can induce viral clearance without causing massive hepatocyte damage. One Phase II study showed that CTL response can be stimulated in patients with chronic HBV infection, who were inoculated with a vaccine that contained an HLA-restricted HBcAg CTL epitope but the antiviral effect was weak (579).

Recommendations for the Treatment of Chronic Hepatitis B

Current therapy of chronic hepatitis B has limited long-term efficacy. Therefore a, careful balance of patient's age, severity of liver disease, likelihood of response, and potential adverse events and complications is needed before treatment is initiated. Except for patients with decompensated cirrhosis, where IFN-α or pegIFN-α are contraindicated, all approved treatments should be considered. For patients who require long-term therapy such as patients with HBeAg-negative chronic hepatitis and those with cirrhosis, lamivudine is not an optimal therapy because of the high risk of drug resistance. For patients with severe acute exacerbation lamivudine or entecavir is preferred as initial therapy because of the slow and sometimes inconsistent antiviral effects of adefovir. The pros and cons of the approved therapies are listed in Table 29.13. Table 29.14 summarizes current recommendations for the treatment of chronic hepatitis B (580,581,582).

Treatment of hepatitis B has evolved at a rapid pace in the past 10 years. With the availability of many new treatment modalities, it is now possible to contemplate combination therapy for hepatitis B. The question is which is the right combination? As our understanding

of hepatitis B improves, it is also possible that future therapies will be tailored to the viral (HBV genotype), immunologic (ALT) and clinical characteristics (stage of liver disease) of the patient.

Table 29.13. Pros and Cons of Approved Treatments of Hepatitis B

	Standard/pegylated IFN	Lamivudine	Adefovir	Entecavir
Route of administration	Parenteral	Oral	Oral	Oral
Duration of treatment	Finite, ~12 m	Indefinite, y	Indefinite, y	Indefinite, y

Viral suppression	Weakest, but has immunomodulatory effects	Potent	Weak, suboptimal response in ~25%	Most potent
Efficacy against lamivudine-resistant HBV	Unclear	NA	Yes	Yes, activity lower than for wild type HBV
Resistant mutations	None identified	15%–30% y 1, 70% y 5	0% y 1, 29% y 5	0% y 1
Side effects	Frequent, may be serious	Negligible	Nephrotoxic, ~5% y 3	Limited safety record carcinogenic in rodents at high doses

IFN, interferon; HBV, hepatitis B virus.

Table 29.14. Recommended Strategies for Patients with Chronic Hepatitis B

HBeAg	HBV DNA[a] > 5 $\log_{10}$ IU/mL	ALT[a]	Recommended strategy
+	+	≤2 × ULN	• Low efficacy of available therapies • Monitor ALT and HBV DNA levels every 3–6 m • Consider treatment if ALT increases to >2 × ULN
+	+	>2 × ULN	• Consider treatment with one of the five approved therapies • Endpoints of treatment: Standard IFN—16 wk PegIFN—48 wk • Oral nucleoside/tide analogs: Lamivudine, adefovir and entecavir, extend therapy for 6 m after HBeAg seroconversion Lamivudine resistance—Adefovir or Entecavir
–	–	≤2 × ULN	• No treatment required • Monitor ALT and HBV DNA levels every 6–12 m
–	+	>2 × ULN	• IFN and pegIFN preferred in young patients because of finite duration of therapy • Adefovir or Entecavir preferred if oral therapy is chosen because of low rates of antiviral resistance with prolonged therapy • Lamivudine only if cost is a significant factor • Goals of treatment: Undetectable HBV DNA by PCR and normal ALT • Duration of treatment: IFN and pegIFN—48 wk Adefovir and Entecavir—>48 wk

+	+	Cirrhosis	• Compensated—Lamivudine or Adefovir or Entecavir • Decompensated—refer for transplant. IFN/pegIFN contraindicated
+	–	Cirrhosis[b]	• Compensated—observe • Decompensated—refer for transplant

[a]Liver biopsy should be considered for patients with HBV DNA of 4–5 $\log_{10}$ IU/mL and ALT 1–2 × ULN, or with fluctuating HBV DNA or ALT levels; those with moderate/severe inflammation, and bridging fibrosis or cirrhosis may benefit from treatment.
[b]Treatment may be considered for patients with cirrhosis even if ALT is normal or HBV DNA is 3–5 $\log_{10}$ IU/mL, especially if decompensated.
HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; ULN, upper limit of normal; IFN, interferon; PCR, polymerase chain reaction.
Modified from Lok A, McMahon BJ. AASLD practice guidelines: chronic hepatitis B http://www.aasld.org/netFORUMAASLD/eweb/docs/chronichep_B.pdf . *Hepatology* 2004;39:857–861.

Hepatitis D

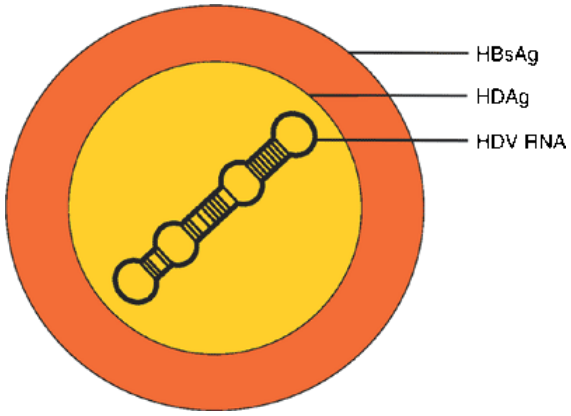
Hepatitis D is caused by a defective virus: The HDV. Although HDV is often referred to as hepatitis delta virus, the term HDV is preferred. HDV can replicate autonomously (583), but the simultaneous presence of HBV is required for complete virion assembly and secretion.

Hepatitis D Virus Structure and Replication

The HDV virion comprises an RNA genome, a single HDV encoded antigen, and a lipoprotein envelope provided by HBV and consisting of the same proteins found in the envelope of the HBV virion. The HDV genome is a small single-stranded circular RNA (1676 to 1683 nucleotides in size) with structural analogies to plant viroids and a high degree of self-complementarity causing the molecule to collapse into a rod-like structure (584). Significant sequence heterogeneity exists among HDV isolates and a classification into three genotypes has been proposed (585), although recent data suggest an even more complex phylogenetic differentiation of HDV into as many as seven clades (586). The only antigen associated with HDV, the hepatitis D antigen (HDAg), is a structural component of the virion: Approximately 70 molecules of HDAg are complexed with the HDV RNA genome to form a ribonucleoprotein structure (587) (Fig. 29.5). Hepatocytes are the only host cells where HDV replicates at very high levels (588) by transcription into a full-length complementary RNA (antigenomic HDV RNA) (584). HDV virion assembly and release is dependent on the simultaneous presence of HBV, which provides the envelope proteins. In the absence of HBV, HDV infection is abortive, unless promptly rescued by HBV (see the following text).

Patterns of Hepatitis D Virus Infection

Due to its dependence upon HBV, HDV infection always occurs in association with HBV infection. The clinical and laboratory findings vary with the type of infection. *Coinfection of HBV and HDV* in an individual susceptible to HBV infection results in an acute hepatitis clinically indistinguishable from classical acute hepatitis B and is usually transient and self-limiting, although a fulminant course was frequently reported among drug addicts (589). The rate of progression to chronic infection is similar to that observed after HBV monoinfection (590). *HDV superinfection* of a chronic HBsAg carrier may present as a severe acute hepatitis in a previously unrecognized HBV carrier, or as an exacerbation of preexisting chronic hepatitis B. Progression to persistent HDV infection is typical (591). A third form of infection is a *helper-independent latent HDV infection*, as reported in the woodchuck experimental model (592) and initially thought to occur in the liver transplant setting.



▪ **Figure 29.5** Schematic representation of the hepatitis D virus (HDV) virion with extensive intramolecular base-pairing of the HDV RNA genome. HBsAg, hepatitis B surface antigen; HDAg, hepatitis D antigen.

Epidemiology of Hepatitis D Virus

Data on HDV epidemiology have mostly been gathered in HBV carriers superinfected with HDV. It was estimated that approximately 5% of the HBV carriers worldwide may be infected with HDV (593). However, substantial changes in HDV epidemiology have occurred in the past 10 years. Improvements in socioeconomic conditions, an increased awareness of the risk of transmitting infectious diseases fostered by acquired immunodeficiency syndrome (AIDS) prevention policy, and aggressive vaccination campaigns against HBV have all contributed to a dramatic decrease in the incidence of HBV infection and the spread of HDV infection, especially in those countries that were previously endemic (594,595,596,597).

Although HDV infection is dependent on HBV infection, the geographical distribution of HDV infection does not parallel that of HBV, as areas endemic for HBV may be almost HDV free. The level of HDV endemicity is partly related to the route of transmission. HDV infection is endemic in the Mediterranean basin, where infection tends to occur early in life and is associated with low socioeconomic status. In the Far East, the prevalence of HDV infection among HBV carriers varies

from 5% in Japan to 90% in some Pacific islands (593). HDV infection is not infrequent in Taiwan (598), being predominantly transmitted sexually, but is rare in Hong Kong where it is largely confined to intravenous drug users (599). In most other Western countries, HDV infection is uncommon and predominantly confined to high-risk groups such as intravenous drug addicts and multiply transfused individuals (e.g., hemophiliacs). Transmission of HDV among HBsAg-positive homosexuals is rare (600). It must be emphasized that the studies mentioned in the preceding text were conducted more than 10 years ago, and the prevalence and risk factors of HDV infection in many parts of the world have not been reexamined.

Diagnosis of Hepatitis D Virus Infection

HDAg elicits a specific immune response consisting of antibodies of the IgM and IgG class (anti-HDV). In HDV infected individuals, the timing of appearance and level of HDV RNA, HDAg and anti-HDV in serum, together with the pattern of HBV markers, allow three HDV-related clinical entities to be identified: Acute HBV/HDV coinfection, acute HDV superinfection of a chronic HBV carrier and chronic HDV infection (Table 29.15). Owing to the dependence of HDV on HBV, the presence of HBsAg is necessary for the diagnosis of HDV infection. The additional presence of IgM antibody to hepatitis B core antigen (IgM anti-HBc) is necessary for the diagnosis of acute HBV/HDV coinfection.

In acute HDV infection, serum HDAg appears early and is short-lived and may escape detection (601). Total anti-HDV antibody appears late in acute infection, and repeated testing is recommended as anti-HDV seroconversion may be the only way to diagnose acute HDV infection in the absence of other markers of HDV infection (601,602). Anti-HDV of the IgM class is transient and delayed if the course of acute hepatitis D is self-limiting, but high-titer and long lasting if HDV infection progresses to chronicity. Although it may be the only serum marker of acute HDV infection (601), anti-HDV IgM lacks specificity, as it is usually found at high titers in chronic hepatitis D. As discussed in the preceding text, the differential diagnosis between HBV/HDV coinfection and HDV superinfection in an HBV carrier depends on the detection of high-titer IgM anti-HBc, found only in patients with coinfection.

Table 29.15. Diagnosis Of Hepatitis D Virus (HDV) Infection

	Acute HDV/HBV coinfection	HDV superinfection of HBV carrier	Chronic HDV infection
HDAg	Early and short-lived	Early, but soon masked by anti-HDV	Undetectable (masked by antibodies)
Anti-HDV, IgM	Transient and delayed	High-titer and long-lasting	High-titer
Anti-HDV, IgG	Late, increasing titers	Late, increasing titers	High-titer
HDV RNA	Early and sensitive marker	Early and sensitive marker	Usually high-level
IgM anti-HBc	Positive	Negative	Negative

HDV, hepatitis D virus; HBV, hepatitis B virus; HDAg, HDV antigen; IgM, immunoglobulin M; IgG, immunoglobulin G; anti-HBc, hepatitis B core antibody.

Development of anti-HDV hampers detection of HDAg further along the course of infection, due to formation of immune complexes. Therefore, in the chronic phase, detection of serum HDV RNA in serum by molecular hybridization or reverse transcriptase-polymerase chain reaction (RT-PCR) is preferred. HDV RNA is an early and sensitive marker of HDV replication in acute hepatitis D (603) and confirms infection in chronic hepatitis D. Furthermore, quantitative determination of HDV RNA is the test of choice for measuring HDV replication in patients undergoing antiviral treatment (604,605).

The detection of intrahepatic HDAg by immunohistochemistry has been proposed as the "gold" standard for the diagnosis of ongoing HDV infection (606). However, at late stages of the disease the detection of HDV RNA in serum by RT-PCR may be more sensitive.

In summary, the diagnosis of HDV infection should be considered in the following clinical situations.

In patients with acute hepatitis B, HDV coinfection should be suspected in patients at high risk, such as intravenous drug users or persons from endemic countries, and those presenting a severe course. Coinfected patients have high titer IgM anti-HBc. Markers of HBV replication may precede or follow those of HDV. Occasionally, patients may have already seroconverted to anti-HBs, for example, at the time of the second phase of a biphasic hepatitis. These patients should still be positive for IgM anti-HBc. Serum HDV RNA is usually positive at presentation, but if this assay is not available, total anti-HDV should be repeatedly tested to document seroconversion.

In acute hepatitis of undetermined origin in a HBV carrier, tests for HDV should be considered to rule out HDV superinfection. As this may occur in previously unrecognized HBV carriers, distinguishing between this condition and acute HBV/HDV coinfection may be difficult.

Testing for HDV should also be carried out in patients with chronic hepatitis B, who live in HDV endemic countries or are at high risk of HDV infection, to rule out simultaneous chronic HDV infection.

This is best achieved by screening for total anti-HDV antibody and then confirmed by staining for HDAg in liver tissues and/or measurement of serum HDV RNA. In chronic HDV infection, HBV replication is usually suppressed, and patients are typically anti-HBe positive.

Pathogenesis of Hepatitis D Virus-Induced Liver Damage

The mechanisms by which HDV induces liver damage (referred to as hepatitis D) are unknown, but are largely thought to be due to the host immune response (584). Overall, the degree of expression of HDV-related liver disease depends on the interplay of HDV-associated (such as HDV genotype) (585,607), host-associated (such as the immune response) and helper virus-associated factors (such as HBV genotype and replication level) (608).

Natural History of Chronic Hepatitis D

The clinical sequelae of HDV infection encompass a spectrum of manifestations from fulminant liver failure to an asymptomatic carrier state. Several studies have suggested that clinical outcome may be related to

the different HDV genotypes (585,609).

Genotype I is more prevalent in the West (610). Here, acute hepatitis D has an increased risk of a fulminant course when compared to acute hepatitis B (589). Once chronic HDV infection is established, it usually exacerbates the preexisting liver disease due to HBV (589) and progression toward cirrhosis is accelerated (611). HDV-associated chronic liver disease may also run an indolent course (612) and asymptomatic HDV carriers have been found in some western countries (613). An unfavorable course toward liver failure has been reported in patients with ongoing HBV and HDV replication (608). It is not clear whether superinfection with HDV accelerates the development of HCC (288).

Genotype II prevails in the Far East, where acute hepatitis D infrequently runs a fulminant course, and chronic hepatitis D seems less progressive (609).

Genotype III is mainly found in South America, where severe outbreaks of acute hepatitis D with a high incidence of liver failure have been reported (585).

Treatment of Hepatitis D

The primary endpoint of treatment is the suppression of HDV replication, which is accompanied by normalization of the serum aminotransferase (ALT) level and amelioration of necroinflammatory activity on liver biopsy. Undetectable HDV RNA in serum and HDAg in the liver document suppression of HDV replication. A secondary, albeit rarely observed endpoint is the eradication of HBV infection, with HBsAg to anti-HBs seroconversion.

The only drug that has been shown to be effective in the treatment of chronic hepatitis D is IFN-α. The mechanism of action of IFN-α in hepatitis D is unclear. IFN-α does not possess in vitro antiviral activity against HDV (614,615). Therefore, the efficacy of IFN-α in patients with chronic hepatitis D may depend on its antiviral activity on HBV or immune modulatory effects. The total number of chronic hepatitis D patients who have been treated with IFN-α and reported in the literature is small. Therefore, it is difficult to draw firm conclusions on the efficacy, optimal dose regimen, and factors predictive of response to IFN-α. Eradication of HDV infection and resolution of liver disease after IFN-α treatment have been reported anecdotally in uncontrolled studies with occasional permanent suppression of HDV replication and improvement of liver fibrosis (616,617,618). However, these favorable effects of IFN-α have not been unequivocally confirmed.

In the largest multicenter trial, 61 Italian patients with chronic hepatitis D were randomly assigned to receive IFN-α in doses of 5 MU/m^2 TIW for 4 months, followed by 3 MU/m^2 TIW for an additional 8 months, or placebo (619). Eight (25%) of the 31 treated patients versus none of the 30 controls had normal ALT at the end of treatment. However, only one patient had normal ALT level at the end of the 12 months follow-up. Fourteen (45%) treated patients were HDV RNA negative at the end of treatment; but a similar proportion (27%) of controls were also HDV RNA negative, suggesting that the "benefit" of treatment may be related to spontaneous fluctuations in HDV viremia. The proportion of HDV RNA-negative patients at the end of follow-up was similar in the two groups (45 vs. 33%). Liver histology also improved with similar frequency in the two groups (57 vs. 36%, *P* = NS). Therefore, the authors concluded that IFN-α therapy did not produce any significant benefit in patients with chronic hepatitis D.

In another study, 42 patients with chronic hepatitis D were randomly assigned to receive two different doses (9 vs. 3 MU TIW) of IFN-α or placebo for 48 weeks (620). Normal serum ALT levels at the end of treatment occurred more frequently in the patients receiving 9 MU doses of IFN-α than in the other two groups (70%, 29%, and 8%, respectively). Complete response (normal ALT level and undetectable serum HDV RNA at the end of treatment) was also more frequent with 9 MU dosing (50%, 21%, and 0%, respectively). Treatment with 9 MU doses of IFN-α was associated with a marked improvement in liver histology. However, none of the patients had sustained clearance of HDV RNA. The authors concluded that high-dose IFN was effective in suppressing HDV replication but that the antiviral effect was not sustained. Five of the ten responders in the 9-MU dose group

had normal ALT levels that lasted for up to 4 years. In a follow-up report they found that ALT normalization correlated with improved hepatic function (621). Compared to treatment with low-dose IFN or placebo, those who received the high dose had a sustained decrease in serum HDV RNA levels, and some patients had HDV clearance. Patients in the high dose group were also more likely to have improvement in histologic activity and fibrosis and survival.

The only feature, which may be associated with an increased likelihood of response, is a short duration of disease (616,617,619). Therefore, although the rate of success is low, it is generally accepted that patients with chronic hepatitis D and active liver disease, as evidenced by elevated ALT levels and/or chronic hepatitis on liver biopsy, should be treated and treated early. The recommended dose regimen is IFN-α 9 MU TIW for at least 12 months. It is very likely that improved results may be achieved by using pegylated IFN-α. Controlled studies are under way.

Lamivudine, a potent inhibitor of HBV replication, has little or no effect on HDV (622,623). There are no data on the efficacy of adefovir or entecavir in patients with HDV infection.

Prevention of Hepatitis D Virus Infection

The mainstay of prevention of HDV infection is vaccination against its helper virus, the HBV. Anti-HBs–positive chimpanzees are protected against experimental HDV infection (624). However, passive prophylaxis with hepatitis B immunoglobulin has not completely prevented reinfection of transplanted livers by HDV.

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Chapter 30
Hepatitis C

Gary L. Davis

Key Concepts

- Hepatitis C virus (HCV) is a single-stranded ribonucleic acid (RNA) virus that replicates at a rapid rate but lacks proofreading ability. As a consequence, it has a high degree of genetic diversity that has led to evolution into several distinct viral genotypes. This genetic diversity affects the biology of the virus, in particular its susceptibility to interferon-based therapy. It may also be an important factor, along with signaling interference and effector modulation, in allowing the virus to evade elimination by the host immune response.
- The serologic test (anti-HCV) is sensitive for diagnosing HCV infection. Molecular tests that measure HCV RNA levels are extremely sensitive and are helpful in confirming infection and managing treatment.
- The incidence of acute HCV infection has fallen dramatically in the United States. The major risk factor for infection remains intravenous drug use.
- Chronic infection develops in 50% to 90% of acutely infected persons; it occurs less commonly in the young. Despite the declining incidence of HCV, the prevalence remains high (3 to 4 million persons) because of this high chronicity rate.
- Both acute and chronic HCV infections are usually asymptomatic. Chronic hepatitis C is a slowly progressive disease but results in significant disease morbidity in only a minority of infected persons. However, because HCV infection is so highly prevalent, chronic hepatitis C is among the most common causes of chronic liver disease in the United States and the leading indication for liver transplantation.
- There is no effective pre- or postexposure prophylaxis for HCV infection. Interferon-based regimens are the only effective treatment for patients with acute or chronic hepatitis, although many patients with acute infection recover spontaneously. Sustained loss of virus is now achievable in more than 50% of patients with chronic hepatitis C who are treated with the combination of long-acting (pegylated) interferons and ribavirin. Patients who have a sustained virologic response to treatment also have significant and persistent histologic improvement.

History

Although our awareness and understanding of viral hepatitis has risen dramatically over the last 4 decades, this is not a new problem. Descriptions of jaundice exist in the literature as far back as several centuries BC and are referenced in the Babylonian Talmud and the writings of Hippocrates (1). The infectious nature of the disease was first recognized in the 8th century BC

by Pope Zacharias (2). However, most of the reports of large population epidemics over the last several centuries were probably due to enteral transmission of what is now known as *hepatitis A*. It was not until the introduction of the practice of inoculation for smallpox vaccination in the 1880s that the percutaneous route of transmission of the disease was recognized (3). Numerous reports of jaundice occurring in patients receiving vaccines or injections for diabetes or syphilis followed during the early 20th century (4,5,6). The first association of blood transfusion with the development of hepatitis was reported in 1943 (7). The landmark studies of Krugman et al. at the Willowbrook State School in New York documented the transmissibility of hepatitis by human plasma (8) and confirmed the long-standing clinical observations that both parenteral ("serum hepatitis") and enteric ("infectious hepatitis") transmission could occur (9). Frustrating and largely unsuccessful efforts to identify the specific agents responsible for hepatitis continued over several decades (10). A serologic marker for hepatitis B was first identified by Blumberg in 1965 (11), although its association with the parenterally transmitted entity then known as *serum hepatitis was* not recognized until 2 years later (12). The recognition of the specific viral agents responsible for hepatitis B and A was made over the next few years (13,14). These discoveries were obviously major breakthroughs, but it quickly became apparent that most cases of hepatitis could not be explained by either the hepatitis A virus or the hepatitis B virus (HBV).

The entity of non-A, non-B hepatitis was formally christened by Prince in 1974 (15). An infectious agent was suspected on the basis of the observations that it was parenterally transmissible to chimpanzees and humans by blood transfusion (16). A series of experiments by Bradley et al. at the Centers for Disease Control and Prevention (CDC) characterized the nature of the infectious agent (17,18). Filtration studies suggested that the agent was between 30 and 50 nm in size. Its infectivity was abolished by chloroform, suggesting the presence of a lipid envelope (17), as well as by formalin, heat (100°C for 5 minutes or 60°C for 10 hours), and β-propiolactone ultraviolet light (18). However, the conventional virologic and immunologic techniques of the time failed to isolate the responsible agent. Scientists at Chiron Corporation, Emeryville, CA, and in Japan used a different tactic based on recently described molecular biologic techniques (19,20). This was based on Bradley's work, which suggested that the non-A, non-B agent was a virus. However, because the genomic nature of this putative virus was not known (although a flavi-like ribonucleic acid [RNA] viral agent was suspected), both deoxyribonucleic acid (DNA) and RNA were extracted for cloning from a large volume of infected serum (19). After extensive ultracentrifugation, which was sufficient to pellet down the smallest of known infectious agents, total nucleic acid was extracted and both RNA and DNA were converted to complementary DNA (cDNA). Restriction fragments were cloned into a recombinant bacteriophage vector to form a cDNA library. These phages were then inserted in *Escherichia coli* capable of transcribing and expressing the encoded peptide, and the resulting products were screened against sera from patients with non-A, non-B hepatitis under the assumption that sera from infected patients should contain antibody against the agent. After more than 1 million clones were screened, 5 clones were found to be reactive. Of these, one clone (5-1-1) was shown to bind not only antibodies present in the serum of patients with non-A, non-B hepatitis but also those in experimentally infected chimpanzees who appeared to seroconvert several weeks after exposure (21). Identification of other clones with overlapping regions of the viral complimentary DNA allowed these investigators to establish the entire viral genome.

This breakthrough led to an explosion of research on this viral agent, now designated as *hepatitis C virus* (*HCV*), and its disease, now called *hepatitis C*. With the development of antibody-based detection systems (see later), HCV was found to be the major cause of non-A, non-B hepatitis (22,23,24). An estimated 170 million persons worldwide are infected by HCV, and it is perhaps the most common cause of chronic liver disease in the United States.

Virology

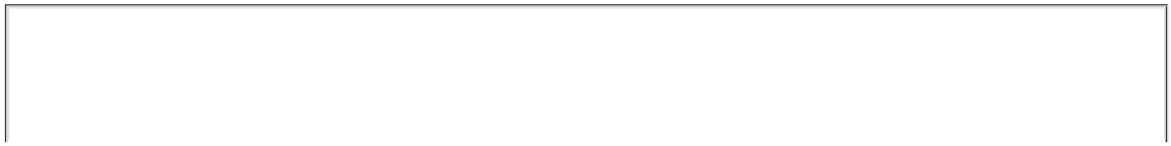
HCV is the only member of the Hepacivirus genus of the Flaviviridae family. The viral genome is contained within a nucleocapsid that is encased in an envelope derived from host membranes into which viral-encoded glycoproteins are inserted (25). Spherical 50-nm viral particles have been identified by electron microscopy (26,27). Two populations of virus appear to exist in serum on the basis of density-gradient analysis (26). The high-density fraction is thought to represent free or immunoglobulin-bound virus, whereas a lower-density fraction appears to be bound to low-density lipoproteins (LDL) (28,29). The pathogenic significance of the latter is discussed later.

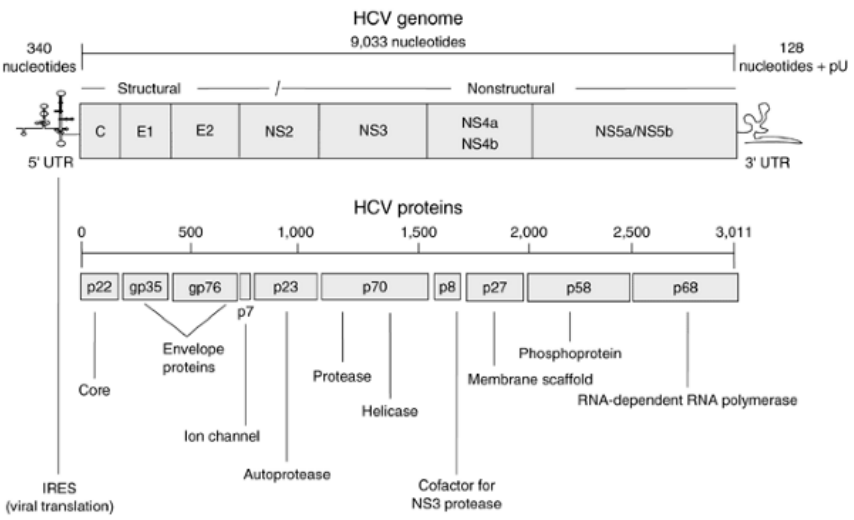
Genomic Organization, Viral Proteins, and Replication

HCV contains a 9.6-kb positive-sense, single-stranded RNA genome that consists of a highly conserved 341-base 5′ noncoding region, a single long open reading frame (ORF) of 9,033 to 9,099 bases, and a 3′ noncoding region. The ORF encodes a polyprotein precursor of approximately 3,000 amino acids (25). This polyprotein is cleaved co- and post-translationally by both

cellular and viral proteases to produce at least ten polypeptides with various functions in replication and virus assembly (30,31) (Fig. 30.1).

On the basis of phylogenetic tree analysis, genetic organization, and hydrophobicity patterns, HCV is related to flaviviruses and pestiviruses and distantly to plant viruses (32). However, HCV has sufficient diversity to justify classification into a separate genus and therefore it has been assigned as the single member of the Hepacivirus genus of the family Flaviviridae (33). The nomenclature of the respective viral proteins is also in accordance with that of the Flaviviridae family (34). Given the factors that influence viral diversity, it is difficult to estimate the age of the HCV by phylogenetic analysis. Nonetheless, ancestors of the oldest genotypes of HCV probably originated in western and sub-Saharan Africa (genotypes 1, 2, and 4) and Southeast Asia (genotypes 3 and 6) (33). Some genotypes and common subtypes evolved later. For example, the subtypes of genotype 2 probably evolved within the last 90 to 150 years. Subtypes 1b likely evolved 60 to 70 years ago, and its global distribution suggests that it disseminated over a short period (33). The recent evolution of genotype 3a (about 40 years) and its high prevalence among intravenous drug users suggest that it may have evolved and spread through the practice of needle sharing in the 1960s (33).





▪ **Figure 30.1** Hepatitis C virus (HCV) genome and encoded polyprotein showing regions and functions. C, core; E, envelope; NS, nonstructural; IRES, internal ribosomal entry site; UTR, untranslated region; RNA, ribonucleic acid; pU, poly-uradine.

Virus Replication

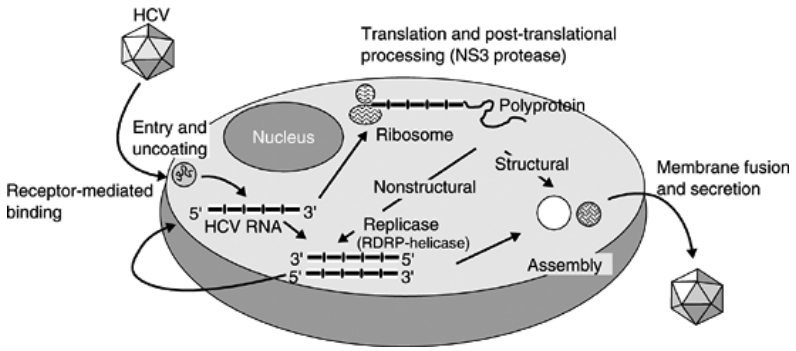
Identification of HCV in the cytoplasm of hepatocytes by immunohistochemistry and in situ hybridization suggests that the liver is the site of viral replication (35,36). This is further supported by the detection of negative-strand HCV RNA, the replication template, in hepatocytes. Although there is some evidence to support extrahepatic reservoirs of virus, including lymphocytes, gut epithelial cells, and the central nervous system, it remains unclear whether these sites simply harbor virus or are actually sites of replication (37,38,39).

Our early understanding of the replication of the HCV was based on the assumption that it must resemble the replication of genetically related flaviviruses. An important breakthrough was the creation of functional subgenomic replicons by Lohmann et al. in 1999 (40). These replicons consisted of subgenomic (incomplete) HCV RNA engineered to express a selectable marker (e.g., neomycin resistance) instead of a virus-related gene, in this case the structural gene region. A heterologous internal ribosomal entry site (IRES) was inserted to facilitate the expression of nonstructural proteins. This replicon was then inserted into Huh-7 human hepatocellular carcinoma cells and transfected cells were selected. Subsequently, replicons derived from other genotypes (Lohmann's original replicon was genotype 1b) and nonhuman and nonliver cell lines have been

used. It became apparent that replicon RNA developed adaptive mutations that allowed it to replicate efficiently in cell culture and these increased replication manyfold (41). One persistent problem with replicon systems was that they did not release viral particles into the media despite containing the full-length HCV genome. The reasons for this were not entirely clear, but it appeared that the adaptive mutation that allowed it to replicate in culture prevented virus assembly. Recently, a full-length replicon derived from a genotype 2a isolate from a Japanese patient with fulminant hepatitis was found to replicate in culture without adaptive mutations and produce infectious particles (42). Despite the engineered and artificial nature of these recombinant constructs, these cell-based replicon systems have provided incredible information on HCV replicative mechanisms and they have also proved useful in screening potential therapeutic agents.

Figure 30.2 illustrates a simplified view of the infection and replication of the HCV in the hepatocyte (43). The mechanisms of HCV attachment and cell entry are still poorly understood. The envelope proteins E1 and E2 contain several *N*-linked glycosylation sites that are conserved and critical for cell entry and protein folding (44). E2 on the HCV particle surface binds to the extracellular loop of the human tetraspanin CD81 with high affinity (45). Although CD81 may serve as an essential attachment receptor for HCV, this binding is not sufficient for cell entry and, in fact, it internalizes ligand poorly (46). Other putative receptors that may participate in HCV entry include the LDL receptor (28,47), scavenger receptor class-B type 1 (SR-B1), liver/lymph node–specific intercellular adhesion molecule 3–grabbing integrin (L-SIGN), and dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) (48). Once bound, the viral and cell membranes fuse, the virus is transported into the cell in an endocytotic vesicle whose acid pH uncoats the virus, releasing the positive strand of the

HCV RNA into the cytosol.

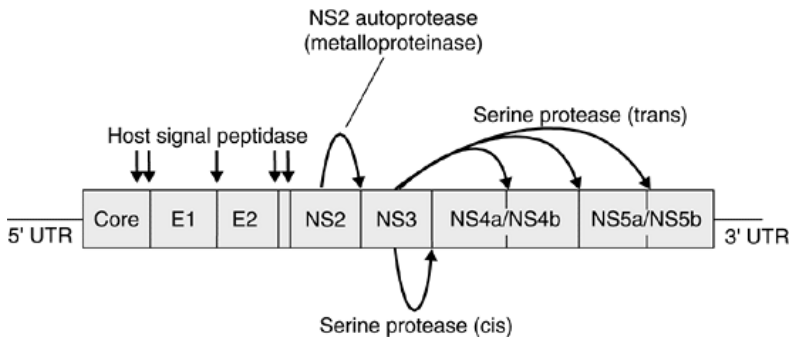


▪ **Figure 30.2** Schematic of the intracellular replication cycle of the hepatitis C virus (HCV). Replication takes place within the cytoplasm and is associated with the endoplasmic reticulum membrane. Assembly occurs in the Golgi. RNA, ribonucleic acid; RDRP, RNA-dependent RNA polymerase.

The 5′ end of the HCV genome contains a highly conserved 341-nucleotide region with a complex secondary structure containing four stem loops or hairpins (49). This untranslated region (UTR) contains critical features necessary for replication and initiation of protein synthesis. First, the last stem loop of the 5′ UTR and the initial part of the core gene functions as an IRES, similar to that first described in poliovirus (50,51). Because HCV lacks a 5′ cap, and therefore does not replicate within the nucleus, it requires an IRES to direct and bind it to ribosomal subunits in the cytoplasm to form the translation complex where HCV replication ensues (51,52,53). This causes conformational changes in the ribosome and binding to eucaryotic initiation factor 3 (eIF3) that serves to position the AUG start codon of the viral structural gene at the decoding site of the endoplasmic reticulum membrane. In this way the positive-strand RNA serves as the messenger for translation of viral proteins.

The ORF of HCV encodes an uninterrupted stretch of 3,011 amino acids, which contains the viral proteins that are necessary for viral replication. This large polyprotein is processed co- and post-translationally by cellular and viral proteases into numerous polypeptides (Fig. 30.3). The proteins encoded by the gene are, in order from the N terminus, the structural proteins, C, E1, E2, and p7, followed by the nonstructural proteins, NS2, NS3, NS4a, NS4b, NS5a, and NS5b. The structural proteins are encoded by nucleotides in the 5′ third of the genome. Three proteolytic activities mediate the cleavages separating structural from nonstructural proteins (Fig. 30.3). Internal leader sequences within the structural precursor direct precise cleavage of the proteins by host signal peptidases within the rough endoplasmic reticulum (54). The structural proteins are produced in

more than 100-fold excess compared to positive- and negative-strand RNA (55).



▪ **Figure 30.3** Hepatitis C virus genome showing protease cleavage sites for corresponding polyprotein. E, envelope; NS, nonstructural; UTR, untranslated region.

The nucleocapsid or core is an unglycosylated basic protein, with RNA-binding capacity controlled by its N terminus and an essential signal for translocation to the endoplasmic reticulum located in the C terminus.

The core protein modulates cell-signaling pathways, transcription, and transformation through its interactions with host proteins. Its sequence is highly conserved and contains several B-cell epitopes (40).

The envelope proteins are highly glycosylated, and some of these glycans play an essential role in protein folding and cell entry (44,56). The proteins are type I transmembrane glycoproteins with tails that serve as endoplasmic reticulum retention signals. They form noncovalently linked heterodimers and disulfide-linked aggregates, the latter being misfolded forms (56). Immune electron microscopic studies have confirmed that these proteins do, indeed, form the structural envelope of the virus (26). Both envelope proteins contain several B-cell epitopes including neutralizing epitopes. The N-terminal residues of E2 exhibit significant amino acid variation between viral genotypes and even within the same host (quasispecies) (57). These variable regions, termed *hypervariable regions* (*HVR1* and *HVR2*), are highly unstructured and are therefore able to tolerate considerable sequence variation (57). Much of this variability may be the result of immune pressure from neutralizing antibodies. Indeed, HVR1 is expressed on the viral surface, and its variability probably contributes in part to the ability of the virus to escape neutralizing antibodies (58). Studies in chimpanzees appear to confirm this because antibody against E2 confers only transient protection against HCV infection (59). p7 is a small protein that is required for replication and may function as an ion channel (60).

In sequence, the nonstructural proteins are then cleaved from the polyprotein. First, the viral autoprotease, a zinc-dependent proteinase encoded by NS2, cleaves the NS2/NS3 junction (61,62). Second, the serine protease, a chymotrypsin-like enzyme encoded by N-terminal third of the NS3 region, cleaves the remaining nonstructural viral proteins at NS3/NS4a, NS4a/NS4b, NS4b/NS5a, and NS5a/5b junctions (Fig. 30.3) (63,64,65). The enzyme is obviously essential to viral replication and contains three highly conserved sites that are thought to represent the catalytic triad of the enzyme (66). The reversible binding of NS4a acts as a cofactor for the protease, allowing the enzyme to assume a more traditional trypsin-like configuration and increasing activity (67). Binding of NS4a to the protease also increases the stability of the enzyme and directs it to the endoplasmic reticulum (68). Interestingly, NS4a from heterotypic isolates can also activate the NS3 protease, although genotype 2 NS4a is a less efficient cofactor (69). In vitro studies have shown that the NS5a/NS5b junction is the most efficiently cleaved site. Protease activity is susceptible to inhibition by its cleavage products (70). The carboxy end of NS3 encodes a nucleotide triphosphatase energy source and another NS3 enzyme, the nucleoside triphosphate-binding RNA helicase, which is thought to facilitate unwinding of the RNA strands during replication (71). The unwinding activities of HCV helicase operate at the 3′ UTR for the generation of the negative-strand RNA replication template and at the 5′ UTR for the production of positive-sense RNA for assembly into infectious viral particles. The NS4 region is cleaved next, producing the NS4a and NS4b proteins. The C terminus of NS4a has been shown to be a cofactor of the NS3 serine protease as described in the preceding text (65,72). It is not essential for the function of the protease but stabilizes it and significantly improves its efficiency (66,67).

The function of NS4b has recently been clarified. It produces vesicular structures in hepatocytes that serve as a membranous web or scaffolding for the replication complex (see subsequent text) (73). NS5a is a phosphorylated protein that has been implicated in RNA binding and interferon (IFN) resistance, at least in some

isolates (74). The region has also been found to contain mutations that allow efficient replication of HCV replicons in Huh7 hepatocellular carcinoma cells (41).

The NS5b region contains the viral RNA–dependent RNA polymerase that elongates HCV RNA strands during replication (75). The enzyme is produced in excess because only approximately 1% of the total polymerase content appears to participate in replication (76). The nonstructural components assemble with the polymerase to form the membrane-associated replication initiation complex. Host cellular factors are involved with this complex as well. This multisubunit, membrane-associated replication complex is called *replicase*. The stem loop structures at the 3′ end of the coding region, but not the entire 3′ UTR, are critical for specific binding of NS5b and probably explain the specificity of the HCV polymerase (77). After binding of the 3′ end of the positive strand to the replication complex, the nascent negative–stranded RNA is elongated and the viral helicase helps separate the RNA strands (71). The negative strand of genomic length can then serve as the template for the production of nascent-positive RNA strands, which can be incorporated into the nucleocapsid that is then encoated with the viral envelope to form the mature HCV virion progeny (78,79,80,81). The HCV particles then bud from the hepatocyte.

The in vivo dynamics of HCV replication have been investigated in humans. On the basis of these studies, it is estimated that HCV replication results in more than 10^{12} viral copies per day (82). Because the half-life of these particles is short (about 2.7 hours), there is a turnover of more than 99% of viral particles daily (82). Turnover is slower (<1% to 33% per day) in infected cells, and the longevity of these hepatocytes is estimated to be 1.7 to 70 days (82).

Genetic Heterogeneity

The HCV RNA polymerase is error prone because it lacks a proofreading exonuclease. As a result of this

low-fidelity system of replication, random uncorrectable nucleotide errors are inevitably introduced, resulting in a heterogeneous population of viral genomes. The spontaneous nucleotide substitution rate of HCV is very high, with a frequency of between 10^{-2} and 10^{-3} substitutions per nucleotide site per year (57,83). Many of these substitutions are lethal and are not reproduced, whereas others do not result in amino acid changes and therefore remain silent. Some amino acid changes may result in increased susceptibility to elimination by the immune system and disappear quickly. Still other substitutions, especially in aggregate over time, can result in amino acid changes that have the potential to alter the biology of the virus. Regardless of the outcome of substitutions at individual sites, the virus as a whole is continually undergoing genetic evolution. As a result, HCV is a heterogeneous virus, with only approximately 70% homology among all known isolates, a level of variability similar to that of other flaviviruses (84). Obviously, the sequence heterogeneity is not evenly distributed throughout the genome. Sequences essential to replication and function are highly conserved because of constraints imposed on nucleotide substitution by secondary structures (85). For example, the 5′ UTR is 95% to 99% conserved, as is the catalytic triad site in the NS3 protease. Less well-conserved regions include core (81% to 91%), envelope (E1, 55% to 75% and E2, 65% to 72%), NS2 (57% to 71%), NS3 (70% to 80%), NS4 (65% to 79%), and NS5 (66% to 79%) (86).

Genotypes

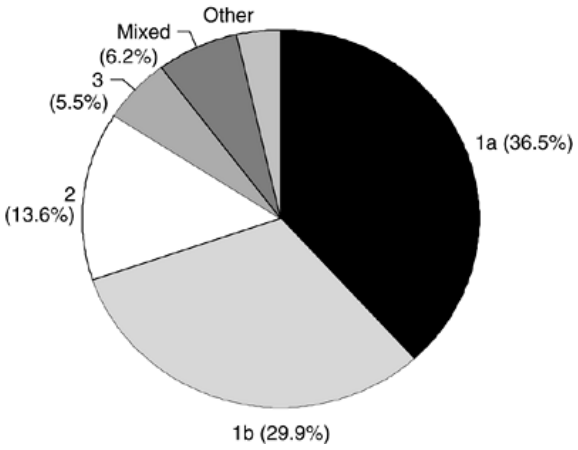
Isolates of a virus are usually distinguished by their genetic relatedness (genotype), much as bacteria, and sometimes viruses, are often separated by antibody reactivity (serotypes). The striking genetic heterogeneity of HCV suggested that the virus might have different genotypes. The validity of this reasoning was supported by a combination of molecular biology and statistical techniques including pair-wise distance determination and phylogenetic tree construction. Different isolates could indeed be classified by their nucleotide variability into genotypes or subtypes (33,86,87). Isolates of the same genotype have an average sequence homology of 95%, with a range of 88% to 100% on the basis of sequencing of relatively well-conserved regions of E1, NS4, or NS5. Subtypes within the same genotype have an average sequence homology of about 80% (range, 70% to 85%). By contrast, different genotypes have sequence similarity of only about 65% (range, 55% to 70%). The distribution of divergence is discontinuous, making the genotypes distinctive relatives rather than a spectrum of variants resulting from random genetic drift (86). A consensus system for HCV nomenclature based on sequence homology in at least two regions confirmed by phylogenetic tree analysis has been established by Simmonds et al. (88). In this system, major genotypes are assigned a number, and subtypes within each genotype are assigned a small letter. The numbering and lettering are assigned in the order in which the type or subtype was originally identified (88). There are six HCV genotypes and more than 100 subtypes. Reports of other genotypes have not withstood scrutiny. HCV genotypes 7, 8, and 9 were proposed several years ago on the basis of unweighted pair-group method with the arithmetic mean analysis of isolates from Southeast Asia (89). However, reanalysis by conventional phylogenetic analysis found these isolates to be subtypes of genotype 6 (88,90). Similarly, reanalysis of isolates reported to represent genotypes 10 and 11 showed that

they were most appropriately classified within types 3b and 6, respectively (91).

There are many methods for determining viral genotype. Although the most accurate is undoubtedly the sequencing of the complete 9,500 nucleotide genome with subsequent phylogenetic tree construction, this is prohibitively expensive and time consuming (92). Furthermore, multiple clones would need to be examined to exclude mixed genotype infections. Therefore, subgenomic genotyping methods have evolved, which are usually based on relatively well-conserved regions of the genome such as the 5′ UTR, core, E1, and NS5b. The nucleotide sequences within these relatively conserved regions are genotype specific, and therefore isolates can be accurately typed regardless of which of these regions is used for analysis (86,87,93). Subgenomic genotypic methods include amplification and region sequencing (94), polymerase chain reaction (PCR) with genotype-specific primers (95), restriction fragment length polymorphism of PCR amplicons (96), differential hybridization including the reverse hybridization line probe assay (LiPA, Bayer Diagnostics, Emeryville, CA) (97), and serologic genotyping (98). The line probe assay is the most popular commercial assay for genotyping. In general, these methods produce equivalent results when confirmed by amplification, sequence comparison, and phylogenetic tree construction (93). However, the method using PCR based on type-specific primers derived from the core region has been shown to be unreliable because its 1b primers frequently react with non-1b isolates, falsely suggesting a mixed genotype infection (95). This technique was one of the first genotyping methods described and was based on the very limited sequence data available at the time. Subsequent modification of the method has apparently resolved these drawbacks (99).

HCV genotype 1 is the most common genotype (40% to 80%) and has a worldwide distribution. Subtypes 1a and 1b are the most prevalent in the United States, each accounting for about a third of cases (Fig. 30.4) (100). Subtype 1b is the most prevalent genotype in Europe, Turkey, Japan, and Taiwan. Genotype 2 is also widely distributed but is less common than genotype 1 (10% to 40%). Type 3 is more common in India, Pakistan, Australia, and Scotland. Type 4 is found predominantly in the Middle East and Africa, type 5 in South Africa, and type 6 in Hong Kong and Macau (86). The geographic differences in genotype

distribution appear to result from several factors. The geographic segregation of genotypes 4 and 6, their phylogenetic distances from more common genotypes, and the diversity within these genotypes all suggest that the major genotypes diverged and evolved in isolation, beginning 500 to 2,000 years ago. By contrast, the common subtypes of genotypes 1 and 2 are more widely distributed and less genetically diverse, suggesting that they evolved more recently, perhaps within the last 50 to 300 years, and were spread by population migration (33,101,102).



▪ **Figure 30.4** Hepatitis C virus genotype distribution in the United States. (See reference 93.)

Quasispecies

Nucleotide substitution over time results in the evolution from a single isolate of HCV to a highly related but heterogeneous population of isolates known as *quasispecies* (103). Sequences begin to diverge about 8 weeks after infection, possibly related to the development of an HCV-specific host immune response (104). Thereafter, the same isolate may evolve into different populations of quasispecies in different patients (105). This probably reflects both some degree of randomness in nucleotide substitution and selective immune pressure. Furthermore, the diversity of the quasispecies probably reflects the duration of infection and the level of replication (103,104,105). Taken together, these factors result in a level of divergence after 11 to 15 years that is just as great among patients infected by the same isolate as it is in unlinked subjects (104). Nonetheless, overall sequence diversity is usually less than 5% in most infected individuals with chronic infection, even after decades of infection. The pathobiologic implications of HCV quasispecies are not well understood but they might play some role in recovery and persistence (106). The implications of quasispecies to the pathogenesis, natural history, and therapy of liver disease are discussed later.

Host Responses and Pathogenesis of Liver Disease

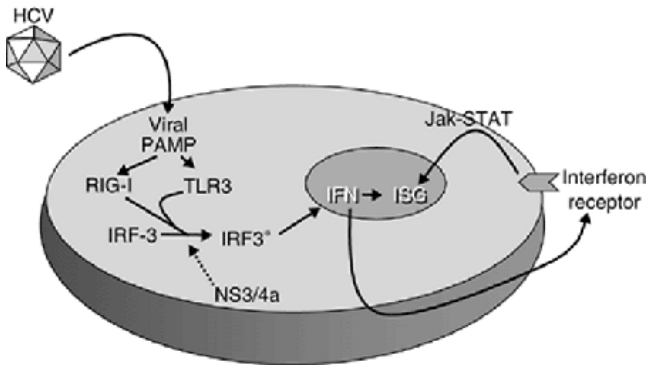
Multiple factors influence the interaction between the HCV virus and the infected host, thereby resulting

in an extremely variable presentation of the infection and liver disease. Viral factors include replication efficiency, expression of viral proteins, virus genotype and diversity, immunoreactivity of viral peptides, and, perhaps, direct liver cell injury. Host factors may include the competence of the innate immune response, local and systemic cytokine production, humoral response, and cellular immune responses. Finally, environmental factors such as alcohol intake and exogenous immune suppression may affect the course of disease. The lack of a small animal or efficient cell culture model has hampered efforts to better define the immunopathogenesis of HCV infection. As a result, most studies have been observational in infected patients. Not surprisingly, the results of these studies are often quite disparate and difficult to interpret because individual variation in the host immune response and subtle methodologic differences in experimental design may influence the findings.

It has become increasingly apparent that persistence of the virus and resistance to IFN-based therapy are related to the host's inability to overcome the viruses' defense mechanisms. The critical factor for virus clearance is the ability of the host to mount and sustain an endogenous IFN response. As described in the subsequent text, the virus works to ameliorate this response and in so doing lessens the chance for early viral clearance and subsequent immune-mediated responses. Viral heterogeneity may also contribute to persistence, but is likely not sufficient in and of itself.

Innate Host Antiviral Defenses

Proteins and nucleic acids of viruses trigger receptors that initiate antiviral mechanisms within the cells (107). The triggers for these mechanisms, termed *pathogen-associated molecular patterns* (*PAMPs*), may include viral envelope proteins or double-stranded RNA segments within the HCV genome (e.g., stems and loops in the UTRs) (108). In the case of HCV, two major pathways exist to initiate host defenses, namely the retinoic acid inducible gene-I (RIG-I) and the toll-like receptors (Fig. 30.5) (109,110). Activation of these pathways by PAMP awakens latent cellular transcription factors that trigger the expression of early response genes in the host cell. IFN regulatory factor-3 (IRF-3) and nuclear factor κB (NF-κB) are the major factors that trigger this response, although other IFN regulatory factors may also be involved (111). This process leads to the secretion of type 1 IFNs in and from the infected cell (112). NF-κB also induces other chemokines and proinflammatory cytokines that modulate the inflammatory response to infection. The secreted IFN then engages its cell surface receptor on both the infected and adjacent cells and activates the Jak-STAT pathway through which dozens of IFN-stimulated genes that produce protein kinases, oligoadenylate synthetase, and other host-protective factors are activated (113). IFN primes the further production of both IFN and the IFN-sensitive genes, thereby amplifying the antiviral responses even further. Finally, IFN modulates the host immune response by enhancing cell surface antigen expression and promotion of immune effector cells.



▪ **Figure 30.5** Schematic of the host innate antiviral responses to hepatitis C virus (HCV) infection. Pathogen-associated molecular pattern (PAMP) bind to retinoic acid inducible gene-I (RIG-I) or toll-like receptor (TLR3) resulting in the phosphorylation (*) and activation of IFN regulatory factor (IRF-3). IRF-3 translocates to the nucleus where it results in interferon (IFN) production. Interferon leads to activation of the Jak-STAT pathway and induction of interferon-sensitive genes (ISGs). NS, nonstructural. (See references 107 and 114.)

The effectiveness of the host response to infection relates in large part to the ability of the infected host to activate and sustain these innate cellular defenses. Some of these responses may be genetically regulated, but it also appears that the levels of IFN signaling, IFN response gene induction, and HCV-induced adaptive immune responses are controlled by the virus itself (Table 30.1) (107). Perhaps most importantly, the NS3/4a protease complex disrupts activation of IRF-3 and NF-κB by the RIG-I and toll-like receptor signaling paths. This reduces the ability of the host to effectively express antiviral defense genes and interrupts

the IFN amplification loop that attempts to suppress HCV replication (114).

Table 30.1. Influence of Hepatitis C Viral Components on Host Antiviral Responses

HCV viral component	Effect on host antiviral response
AUGMENT HOST RESPONSE	
HCV viral particle	Binding to dendritic cell activates host IFN production and immune induction
dsDNA	Triggers IFN and activates PAMPs
Core	Activates PKR

NS5a	Activates STAT3
INHIBIT HOST RESPONSE	
E2	Inhibits PKR
Core	Increased suppressor of cytokine signaling blocks Jak-STAT signaling
NS3/4a protease	Disrupts RIG-I and TLR3 signaling for IRF-3 activation
NS5a	IFN antagonist
	Induces IL-8 that interferes with IFN actions
	Inhibits PKR signaling and IRF-1 action
HCV genomic sequences	RNase L recognition sites reduced in genotype 1

HCV, hepatitis C virus; IFN, interferon; dsDNA, double-stranded deoxyribonucleic acid; PAMP, pathogen-associated molecular patterns; PKR, protein kinase R; NS, nonstructural; E2, envelope protein 2; RIG-I, retinoic acid–inducible gene-I; TLR, toll-like receptor; IRF, interferon regulatory factor; IL, interleukin; RNase, ribonucleic acid proteinase.

Acquired Immunity

The host immune response to HCV infection is composed of both a nonspecific immune response, including endogenous cytokine production (see preceding text) and natural killer (NK) cell activity, and a virus-specific immune response, including both humoral and cellular components (Table 30.2). Antibodies to HCV structural and nonstructural proteins develop late during acute infection and form the basis for the detection assay of the host's exposure to the virus (See "Diagnostic Tests"). A variable cellular immune response also occurs early during acute infection and results in the emergence of $CD4^+$ and $CD8^+$ cells that recognize and respond to processed HCV antigens.

Humoral immune response

Evidence for activation of the host humoral response in HCV infection includes the presence of hepatic lymphoid aggregates containing activated B cells (115), elevated levels of the B-cell–activating interleukin-4 (IL-4) (116), and a B-cell–mediated response with the production of antibodies to several structural and nonstructural polypeptides (117). Antibodies to HCV peptides form the basis of current diagnostic assays (See "Diagnostic Tests"). However, the role of this humoral immune response in the control of infection and pathogenesis of liver disease is still largely unknown.

Antibodies to HCV antigens develop in most acutely infected patients within 4 to 8 weeks of the onset of infection. However, their relatively late onset, low level during acute infection (117,118), decrease and occasional disappearance when infection resolves (119,120), and persistence at higher levels when infection persists raises questions about their relevance in control of infection. Nonetheless, the humoral response may influence liver disease associated with HCV infection in other ways such as extracellular viral neutralization. Antibodies against envelope proteins often have neutralizing capability that could prevent viral entry into uninfected cells. Antibodies against conserved epitopes of the HCV envelope proteins (E1, E2) are found in more than 90% of patients with chronic HCV infection (117). However, the persistence of infection in most patients with anti-E1/anti-E2 suggests that either the antibodies do not have sufficient neutralization ability or the target is not relevant to viral persistence. There is no doubt that neutralizing antibodies can be raised, and these can even neutralize infectious virus ex vivo, thereby preventing infection (121,122). However, the in vivo neutralizing antibody response is usually weak and short lived. Farci et al. demonstrated that the infectivity of plasma can be neutralized by serum obtained from the same chimpanzee 2 years after infection but not by serum obtained 11 years later, presumably because of the evolution of quasispecies (123). Similarly, in other experiments, convalescent animals could be reinfected by either heterologous or autologous challenge (124). Therefore, it appears that

although neutralizing antibodies are formed, evolution of the virus may allow escape from this response. This is not unexpected because the envelope proteins are not highly conserved. E2 contains two HVRs in which nucleotide substitutions are largely unconstrained and wide genetic differences evolve. It has been suggested that antibodies to these regions (especially HVR1) neutralize existing strains of the virus and drive genetic drift, but this is controversial and most recent evidence weighs against such an effect (125,126,127,128).

Table 30.2. Summary of Components of the Hepatitis C Virus

HUMORAL
Activated B cells in lymphoid aggregates
Peripheral expansion of $CD5^+$ B-cell population
Antibodies to structural and nonstructural peptides
No evidence for antibody-dependent cellular cytotoxicity
CELLULAR
Innate
Inflammatory cytokine and IFN release
Natural killer cell activation
Natural T-cell activation
CD4 lymphocytes
Early response
Augments antibody production by B cells
Primarily act peripherally
Possible role to control infection and protect liver
Stimulates CD8 cells
Later response
Compartmentalization to liver
May target different HCV peptides from peripheral CD4
CD8 lymphocytes
Compartmentalization in liver
Predominant infiltrating lymphocyte
Probable role in controlling replication and causing liver injury
Activity possibly results from upregulation of adhesion molecules to recruit CD8 cell upregulation of Fas proteins may facilitate CD8 target killing induction of local cytokine release (IFN-γ, TNF-α)
Cytokine response
T_H1 response early in infection and persists locally in liver
T_H2 response may be a peripheral autoregulatory response to T_H1 activation

HCV, hepatitis C virus; IFN, interferon; TNF, tumor necrosis factor.

Antibodies may also direct the destruction of their bound target through activation of other mechanisms, specifically the complement-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). However, for these antibodies to contribute to cell injury, they must recognize HCV antigens on the hepatocyte cell membrane. Although HCV antigens (core, E1, E2, NS3, and NS4) have been detected in the cytoplasm of infected hepatocytes, membranous antigens have not been observed (129). Even when intracellular expression of HCV viral proteins is driven to very high levels by a recombinant vaccinia system, neither HCV antigens nor immunoglobulins could be detected on the cell membrane (130). These data suggest that ADCC is unlikely to play an important role in mediating hepatocellular damage.

Although evidence suggesting a role for the humoral response in HCV liver disease is still lacking, the antibody response may be associated with other manifestations of infection (See "Extrahepatic Manifestations"). It has been suggested that binding of HCV to the CD81 receptor on B cells could activate these cells, thereby facilitating the production of antibodies (131,132). Indeed, patients with chronic HCV infection commonly develop autoantibodies (133). One of these, the antibody to the human-derived epitope GOR, appears to result from molecular mimicry between the HCV core sequence and GOR (134). This is supported by the rarity of anti-GOR in patients infected with HCV genotype 3, which has amino acid differences within the proposed molecular mimicry site. However, there is no evidence to suggest that autoantibodies have clinical significance or a role in disease pathogenesis (135).

Humoral activation during HCV infection is not limited to antibody production. More than half the patients with chronic HCV infection show expansion of $CD5^+$ B lymphocytes in peripheral blood (136). Again, this

might be related to binding of HCV to B-cell CD81 receptors (131,132). Activation of CD5 B cells has previously been associated with autoimmune diseases such as rheumatoid arthritis (137), and it is possible that a similar mechanism plays a role in the development of B-cell lymphomas in patients with HCV infection (138). HCV is also associated with the development of mixed essential cryoglobulinemia, in which deposition of immune complexes composed of inmmunoglobulin G (IgG) and rheumatoid factor precipitates in small blood vessels (139). This appears to be due to antigen-driven benign proliferation of B cells. The mechanism may be through the CD81 mechanism discussed earlier or due to impaired ability of hepatocytes to endocytose HCV–very low density lipoprotein (VLDL) complexes containing apo E2 through the LDL receptor. Retention of the complexes in the circulation may then stimulate rheumatoid factor production (139).

Cellular immune response

The cellular immune response to viral infection involves innate nonspecific mechanisms, such as those described in the preceding text and NK cell activity, and adaptive antigen-specific mechanisms, including cytotoxic T lymphocytes with accompanying inflammatory cytokine release (Table 30.2). Although nonspecific cellular responses may act early to limit some infections, eradication of infection appears to also require specific and classical $CD4^+$ and $CD8^+$ cytotoxic T-lymphocyte (CTL) responses. However, as previously described, activation of endogenous antiviral mechanisms and adaptive cellular responses may be linked.

In contrast to the humoral response triggered by the binding of unprocessed extracellular antigens to B-cell immunoglobulin receptors, the cellular (T-cell) immune response is triggered by peptides that have been processed within the cell cytoplasm and expressed on cell membranes in conjunction with a major histocompatibility complex (human leukocyte antigen [HLA])

molecule. Processed peptides are generally presented to $CD8^+$ T cells by HLA class I molecules, which are expressed on virtually all cells, or to $CD4^+$ T cells by the HLA class II molecules, which are found on specialized antigen-presenting cells. Although the subsequent events that lead to the death of the infected presenting cells are not entirely clear, both direct cytolysis and secreted antiviral factors (tumor necrosis factor-α [TNF-α] and IFN-γ) can be implicated.

Innate cellular response

The liver has a dense population of lymphoid cells representing approximately 10% of all lymphocytes and 35% of T lymphocytes in the host (140). These cells comprise a highly regulated immune environment within the liver for monitoring and implementing responses to foreign stimuli. The innate response is the earliest phase of immune defense to viral infection and helps regulate the subsequent adaptive responses of the host. Over a third of hepatic lymphoid cells are NK cells that can be nonspecifically activated by a variety of signals and are able to kill targets in the absence of antibody or antigenic stimulation. Histocompatibility antigen expression appears to inhibit NK activity. In the case of viral infection, downregulation of endogenous class I histocompatibility antigens occurs, possibly to assist the virus in evading CTLs, and this may result in early NK cell activation (141). NK cells appear to exert their activity against HCV by releasing IFN-γ (142), but it appears that they are only able to control acute infection at low levels of replication (143). Natural T (NT) cells (also called innate T cells) are $CD3^+$, common in the normal liver, and prevalent in HCV-infected liver (144). These cells produce IL-4 and probably play a role in modulating both early and subsequent immune responses. Finally, memory HCV–specific T cells are sometimes present before HCV exposure because of cross-reacting antigens. For example, immunodominant HCV-specific CTL response to the NS3 epitope can be induced by influenza A infection because of sequence homology between NS3 and the neuraminidase protein (145). Although these latter two cell types are active in HCV infection, their role in the initial control of infection is not known.

$CD4^+$ T-lymphocyte response

The $CD4^+$ T-cell response to viral proteins is critical for host protection. It occurs early, augments antibody production by B cells, and is a prerequisite for subsequent $CD8^+$ T-cell responses, including those that are specific for virus-infected cells (146,147). Therefore, its role has typically been viewed as a protective one. $CD4^+$ T-cell responses to viral infection have traditionally been determined by measuring the ability of these peripheral lymphocytes to proliferate or produce IFN-γ when exposed to viral proteins. No one viral antigen is responsible for this $CD4^+$ response, although peptides derived from core and NS4 result in the strongest proliferative responses (148). Interestingly, proliferative $CD4^+$ responses are most robust in infected individuals in whom acute infection resolves (149), who have persistent infection without histologic evidence of liver damage (150), or who have chronic hepatitis that responds to IFN (151). These observations suggest that a vigorous $CD4^+$ response to HCV infection provides early control of infection and protects against subsequent hepatocellular damage.

The studies of peripheral blood proliferative responses must be considered with some degree of skepticism because these circulating $CD4^+$ cells may not accurately reflect the immunologic climate at the

site of infection. Indeed, it appears that HCV-specific proliferative responses tend to home in on the liver once chronic infection is established (152). Although studies of intrahepatic $CD4^+$ responses have been limited, proliferative responses to core, E1, and NS4 have been reported (152,153). Several striking differences from peripheral $CD4^+$ responses have been noted. First, the reactive $CD4^+$ clones do not always react to the same HCV peptides that are recognized peripherally (152), and second, proliferative response appears to correlate with more active liver disease (153). Finally, intrahepatic $CD4^+$ T cells differentiate into both T-helper cell 1 (T_H1) (IFN-γ) and T_H2 (IL-4) populations, although the former predominate (154).

In summary, $CD4^+$ cells play a role early in infection, perhaps by local cytokine production, providing help for B cells and protection of the hepatocyte from injury. $CD4^+$ cells seem to compartmentalize to the liver after the resolution of the acute phase, produce predominantly T_H1 cytokines, and may even generate or directly develop HCV-specific CTL activity (146). However, $CD4^+$ activity is typically weak in patients with chronic infection, and this may impair the development of an effective $CD8^+$ response. It has been proposed that HCV core binds to the complement receptors of $CD4^+$ T cells and dendritic cells, thereby impairing the production of IL-2 and IFN-γ that are required to generate T-cell effector functions (155).

$CD8^+$ T-lymphocyte response

The $CD8^+$ arm of the cellular immune system has been shown to be important in the control of viral infections and pathogenesis of cell injury in vivo. Several lines of evidence suggest that these cells also play an important role in HCV infection. First, immunophenotyping studies have demonstrated that a significant proportion of the activated cells in the livers of patients with chronic hepatitis C are $CD8^+$ T lymphocytes (156,157). Second, expression of adhesion molecules,

one pathway for recruitment and priming of T cells, is upregulated in the inflamed hepatic portal tracts (158). Third and most important, HCV-specific cytotoxic $CD8^+$ T lymphocytes have been isolated from both liver and peripheral blood in a significant proportion of patients with chronic HCV infection (159,160,161). These cells are restricted by HLA class I molecules, suggesting that HLA subtypes might present different processed viral peptides to lymphocytes and affect the severity of cell injury. Indeed, target epitopes from within both structural and nonstructural regions have been identified (162,163,164). The immunodominant CTL epitopes are most commonly located within the HCV structural antigens (core/E1/E2); CTL responses to nonstructural regions occur in a smaller subset of patients (161). However, multiple epitopes may be targeted by the same patient, and the magnitude of the CTL is variable both within the same patient and between different patients (165). Interestingly, HCV-specific CTL cannot usually be detected in peripheral blood without HCV-specific stimulation (161). The estimated frequency of HCV-specific CTL in the peripheral blood mononuclear cells of patients with chronic hepatitis C is only 1 per 30,000 to 1 per 300,000 (162). This tissue-dependent prevalence suggests compartmentalization of HCV-specific CTL activity, similar to what was described earlier for $CD4^+$ cells (152). Of course, it is not surprising that the liver, the primary and perhaps only site of HCV replication, is the recruiter of HCV-specific CTL. However, although HCV-specific $CD8^+$ cells may be more frequent in the liver, they appear to be dysfunctional, with impaired ability to secrete IFN-γ compared to peripheral cells (166). The mechanism for this is not clear but could be related to the viral inhibition of IFN production (107).

Nonetheless, HCV-specific CTL appears to have an important role in the control of viral replication and promotion of hepatocellular damage in chronic HCV infection. Using an in vitro protocol to expand liver-derived $CD8^+$ cells without HCV-specific stimulation, Nelson et al. (161) have measured HCV-specific CTL activity in bulk-expanded $CD8^+$ T cells isolated from liver. Patients with measurable HCV-specific CTL activity had higher serum alanine aminotransferase (ALT) levels and more histologic activity in the liver biopsy. Furthermore, these patients also had significantly lower HCV RNA levels, suggesting that HCV-specific CTL activity may be important in regulating HCV replication. A similar role for virus-specific CTL has also been reported for lymphocytic choriomeningitis virus (LCV) infection (165). In fact, in LCV infection, $CD8^+$ CTL may lead to viral clearance through direct lysis of infected cells or cytokine-mediated viral inhibition (167). Current evidence suggests that HCV-specific T-cell responses do not lead to viral escape mutations (168).

The means by which the HCV-specific CTL mediates these effects remains largely speculative. CTL may reduce viral replication by the local production of lymphokines such as IFN-γ and TNF-α (135,136,137), although some studies have shown that this response is impaired compared to the response to other viruses such as influenza (166). Apoptosis may be the most important mechanism of hepatocyte death by CTL (169). This is likely to occur by a variety of apoptotic pathways. CTLs can be directly cytopathic to their targets by inducing the formation of pores in the target cell membrane. This occurs through a sequence of events resulting in the secretion of the pore-forming protein perforin, a series of granule serine proteinases (granzymes), and other molecules (170). CTL can also induce apoptosis by facilitating the interaction of the Fas/Apo 1 antigen with its receptor ligand on the surface of the activated $CD8^+$ cells (171). In fact, hepatic expression of c-Fas is increased in patients with chronic HCV infection, and Fas-bearing cells are more sensitive to CTL killing (172,173). HCV core expression may be important in

making cells susceptible to Fas-mediated apoptosis (174).

Cytokine response

The high replication rate of HCV and the large number of infected hepatocytes present a formidable challenge to the cellular immune system. The fact that this infectious burden exceeds the capacity of the CTL response is apparent from the persistent nature of the infection. However, other mechanisms may assist in controlling the infection. Cytokines are regulatory molecules that play an important role in orchestrating several physiologic and pathologic processes. Cytokine responses are referred to as T_H1, T_H2, or the inactive T_H0, after the original description of the cytokine profiles produced by subsets of the $CD4^+$ T_H cells (175). T_H1-like responses include IL-2, TNF-α, and IFN-γ secretion and are required for CTL generation and NK cell activation during the host's antiviral immune response. T_H2-like responses produce IL-4 and IL-10, which help augment antibody production and inhibit the development of the T_H1 response (176).

Patients with chronic HCV infection have an activated T-cell response pattern and have been reported to have elevated serum levels of both T_H1 and T_H2 cytokines (177,178). Peptide stimulation of either peripheral blood- or liver-derived HCV-specific T-cell clones results in a predominantly T_H1 cytokine response with release of IFN-γ and TNF-α (179). Furthermore, IFN-γ and IL-2 messenger RNA (mRNA) levels are increased in the livers of patients with chronic hepatitis C, suggesting that these cytokines are produced locally by resident $CD4^+$ cells (180). The levels of IFN-γ and IL-2 mRNA correlate with fibrosis

and portal inflammation, suggesting that T_H1 cytokines play a role in mediating hepatocellular damage. To further support this hypothesis, elevated plasma levels of TNF-α appear to be associated with more severe hepatocellular damage (181). However, others have reported a predominantly T_H2 profile with elevated serum IL-4 and IL-10 levels and some T_H2 cell markers in hepatic inflammatory infiltrates (116,177). IFN treatment appears to reduce this T_H2 cytokine response in parallel to the reduction in viral levels (182). Given the ability of IL-4 and IL-10 to inhibit immune cell function, T_H2 cells may provide an autoregulatory mechanism through which the host is able to partially offset the potentially detrimental effects of the T_H1 response.

From the existing data, it appears that the T_H1 response is activated in the liver in response to HCV infection. This may be an attempt to control viral replication, and its persistence results in hepatocyte injury. The T_H2 cytokine response probably represents an autoregulatory response that originates outside the liver and attempts to confine the T_H1 response to the site of infection (the liver) to prevent systemic effects.

Direct viral cytopathicity

Some viruses can kill cells directly without invoking immune-mediated pathways. This may occur through the ability of some viruses or viral gene products to increase lysosomal permeability, alter cellular membranes, or interfere with normal cellular functions such as the synthesis of cellular macromolecules. Characteristic morphologic alterations of cellular architecture such as cell rounding and shrinkage and nuclear pyknosis suggest a cytopathic effect.

It has been difficult to determine whether HCV is directly cytopathic because cell culture systems that allow replication of unmodified HCV have not yet been perfected. However, several lines of evidence support a cytopathic role for HCV. First, other members of the Flaviviridae family, such as the yellow fever virus, cause direct cytopathic injury to infected cells (183). Second, histologic examination of HCV-infected livers occasionally reveals dying hepatocytes without adjacent inflammation (184,185). Third, serum aminotransferase levels and hepatic inflammation decline relatively parallel to viral levels during treatment with antiviral agents such as IFN (186). And finally, some studies have found a correlation between serum HCV RNA levels and the degree of hepatocellular damage (187,188). In fact, high-level cellular expression of HCV has been seen in some patients with severe hepatic injury. This was first reported in an immunosuppressed heart transplant recipient who acquired acute HCV infection from the donor organ (189), but a similar picture has since been reported in other immunosuppressed patients particularly after liver transplantation (190,191). In many of these cases an unusually high proportion of the liver cells contain HCV, and liver biopsies reveal an atypical histologic picture of pericellular fibrosis, marked intracellular cholestasis, and only mild inflammation. This pathology is similar to the fibrosing cholestatic hepatitis sometimes seen in highly viremic immunosuppressed patients with chronic hepatitis B (192). If HCV is indeed cytopathic at high levels, one would anticipate that high-level expression of HCV proteins in vitro might alter hepatocellular structure or function. Indeed, evidence supporting this hypothesis was recently provided in cell lines expressing high levels of HCV structural proteins. These cells showed mitochondrial and endoplasmic reticulum proliferation, distension of the endoplasmic reticulum, and hepatocellular ballooning similar to those seen in the infected transplant recipients described in the preceding text (193).

On the other hand, there is also evidence to suggest that HCV is not directly cytopathic. In the overwhelming majority of patients, particularly immunocompetent patients, biochemical or histologic markers of disease activity do not correlate with serum viral levels or the amount of HCV RNA or antigen

in the liver (194,195). In fact, many patients with HCV infection have persistently normal serum ALT levels and minimal liver injury despite the presence of detectable HCV RNA in serum (196). Furthermore, a transgenic mouse model with high-level expression of HCV structural proteins does not demonstrate cytopathic changes in the liver, and this has called into question the cell culture findings discussed in the preceding text (197). Taken together, these observations suggest that direct cytopathic injury due to expression of HCV structural proteins is not typical but might be possible when the unusually high levels of the virus exceed a certain threshold. However, such injury is not predictable and therefore is probably dependent on factors other than simple expression of virus or protein.

Diagnostic Tests

Screening and Supplemental Antibody Tests

The screening diagnostic test for HCV infection is a sensitive and unique enzyme immunoassay (EIA) in which antibodies to several different viral antigens (anti-HCV) are simultaneously detected. This commercially available assay is simple to perform, reproducible, and relatively inexpensive. A supplemental recombinant antigen immunoblot assay (RIBA) is available to resolve false-positive results in the screening tests, although this assay is rarely necessary today. Three versions of the anti-HCV EIA test have been developed over the last

several years. The antigens included in the tests and the sensitivity of the different versions are important to recognize for historical purposes (Tables 30.3 and 30.4).

Table 30.3. Hepatitis C Virus Peptides in Immunodiagnostic Assays

Peptides	EIA-1	RIBA-1	EIA-2	RIBA-2	EIA-3	RIBA-3
5-1-1 (NS4)		X		X		
C100-3 (NS3-4)	X	X		X		X
C33c (NS3)				X		X
C200 (fusion C100/C33)			X		X	
C22-3 (core)			X	X	X	X
NS5					X	X

EIA, enzyme immunoassay; RIBA, recombinant antigen immunoblot assay; NS, nonstructural.

The first-generation test (EIA-1), introduced immediately after the discovery of HCV (21), was an important first step in diagnosis, particularly for the screening of blood donors to reduce post-transfusion hepatitis (22,198). However, because the assay used only a single target antigen, it lacked sensitivity. In fact, only 80% of infected patients were antibody positive by this test (199). However, false-positive results were also common. Therefore, the supplemental RIBA test that used the same and sometimes additional HCV peptide antigens affixed to a solid phase was often required to resolve the specificity of the EIA result (Table 30.3) (200). This assay allowed visualization of the peptide target of the reactive serum of the patient, confirming or refuting whether the serum reacted specifically against HCV antigens. The importance of the supplemental RIBA test was particularly evident in a low HCV prevalence setting, such as with screening healthy blood donors. In this situation, 50% to 70% of the positive EIA-1 results were subsequently shown to be false-positives when tested by the supplemental assay (199). It is important to recognize that the RIBA assay is not as sensitive as the EIA test and, therefore, should not be used for screening purposes. It is most appropriately used to confirm positive EIA results in low-prevalence settings such as with healthy blood donors and anti-HCV–positive patients with normal ALT levels. Because most infected patients are EIA positive, confirmation by RIBA is generally not necessary when the serum ALT level is elevated or risk factors for infection are present.

Table 30.4. Sensitivity and Predictive Value of Serologic Tests for Hepatitis C Virus Infection

Assay	Sensitivity (%)	Positive predictive value		Time to positive (wk)[a]
		Low prevalence (%)	High prevalence (%)	
EIA-1	70–80	30–50	70–85	16
EIA-2	92–95	50–60	88–95	10
EIA-3	97	25	98	7–8

[a]Time after infection.
EIA, enzyme immunoassay.
Adapted from Carithers RL, Marquardt A, Gretch DR. Diagnostic testing for hepatitis C. *Sem Liver Dis* 2000;20:159–172.

The first-generation anti-HCV test was replaced in 1992 by a multiantigen test, EIA-2 (201), which had HCV antigens from the core and third and fourth nonstructural regions (NS3 and NS4) (Table 30.3). This provided greater sensitivity and specificity. EIA-2 detects anti-HCV in at least 95% of infected patients, which is a great advantage over EIA-1 (199). Because the test is more sensitive, it is capable of identifying new infections at an earlier time (mean, 10 weeks postinfection vs. 16 weeks with EIA-1) (201). Finally, the more sensitive EIA-2 improved the identification of potentially infectious blood donors and resulted in a further reduction in the incidence of post-transfusion hepatitis C (202,203). The use of multiple target peptides also appeared to improve the specificity and reduce the false-positive rates in low-prevalence groups to 40% to 50% (204,205).

EIA-3 was approved for screening blood products in the United States in 1997. Although the EIA-3 test contains reconfigured core and NS3 antigens, as well as an NS5 peptide not included in earlier versions of the assay (Table 30.3), these changes result in only a small increase in sensitivity (206). The major advantages of EIA-3 are earlier identification of acute infection (7 to 8 weeks in up to a third of patients) and fewer false-positive tests in low-prevalence populations (204,206).

A third-generation supplemental test (RIBA-3) consists of the antigens added to EIA-3 and the peptides already present in RIBA-2 (Table 30.3). RIBA-3 is more specific than RIBA-2 (correlates better with HCV PCR) and has fewer indeterminate results (207,208).

Measurement of Viral Ribonucleic Acid

Detection of HCV RNA in blood by a highly sensitive assay is important for confirming the diagnosis of hepatitis C and for assessing the antiviral response to IFN therapy. Qualitative HCV RNA tests such as the reverse transcription PCR (RT-PCR) or transcription-mediated amplification (TMA) are particularly useful as a diagnostic tool in confirming the presence of infection in special situations such as in immunocompromised individuals in whom antibody is less likely to be present or in seropositive patients with a normal serum ALT level (209). In the latter the HCV infections may either have resolved (i.e., HCV RNA negative) or be viremic (HCV RNA positive) despite normal liver enzyme levels. Although the natural history and management of such patients remains controversial, testing of HCV RNA seems medically prudent so that patients with viremia may be counseled about the potential risks of virus transmission, disease progression, and the possibility of antiviral treatment. Qualitative testing is also important to confirm the clearance of HCV after antiviral therapy. Quantitative HCV RNA tests, by either PCR or signal amplification, are used to guide antiviral therapy. In this role, HCV RNA testing can provide a reference point before initiation of therapy. Monitoring HCV RNA during therapy allows early prediction of response (See "Prevention and Treatment").

Qualitative hepatitis C virus ribonucleic acid tests

Qualitative assays for HCV RNA simply determine whether the virus is present. These tests have the potential to be extremely sensitive (Table 30.5). There are two methods that are widely available in commercial laboratories for testing clinical samples—RT-PCR and TMA. Both methods require extraction of nucleic acid from the specimen. RT-PCR then involves reverse transcription of the sample RNA to its cDNA using specific primers based on highly conserved sequences of the virus genome. The DNA product is then repeatedly amplified with a bacterial DNA polymerase until the amount of product reaches a level that can be detected by autoradiography, ethidium bromide staining, or colorimetric testing. Most of the early RT-PCR assays for HCV RNA were designed by individual laboratories (so-called home-brew tests),

had limited sensitivity (1,000 to 2,000 copies per milliliter), and were subject to error related to lack of standardization and sample contamination (205). The development of RT-PCR tests by commercial diagnostic laboratories has employed standardized methodology, easier assay formats, and routine assay controls. These measures have eliminated many of the problems experienced with the early assays. The widespread availability of commercial PCR tests for HCV RNA such as the Amplicor assay (Roche Diagnostics, Nutley, NJ) has made diagnosis and clinical management of hepatitis C much easier.

Table 30.5. Characteristics of Commercially Available Hepatitis C Virus Ribonucleic Acid Assays

Test	Manufacturer	Method	Application	Dynamic range (IU/mL)[a]		Conversion factor (copies/IU)
				Lower limit	Upper limit	
Amplicor	Roche	PCR	Qualitative	42	N/A	N/A
UltraQuant	NGI-LabCorp	PCR	Qualitative	30	N/A	N/A
Versant-TMA	Bayer	TMA	Qualitative	5	N/A	N/A
Amplicor Monitor 2.0	Roche	PCR	Quantitative	600	500,000	2.4
SuperQuant	NGI	PCR	Quantitative	60	1,500,000	3.4
QuantaSure[b]	NGI	PCR	Quantitative	2	2,000,000	2.5
Versant Quant 3.0	Bayer	bDNA	Quantitative	520	8,300,000	4.8
LCx	Abbott	kLCR	Quantitative	23	2,300,000	4.3
Heptamax	Quest	kPCR	Quantitative	50	50,000,000	2.7
TaqMan ASR	Roche	kPCR	Quantitative	25	5,000,000	0.6

[a]Limits of detection are approximated on the basis of published data.
[b]This test increases the lower limit of detection by centrifugation of a larger sample.
PCR, polymerase chain reaction; N/A, not applicable; NGI, National Genetics Institute; TMA, transcription-mediated amplification; bDNA, branched deoxyribonucleic acid; LCx, ligase chain reaction; kLCR, kinetic ligase chain reaction; kPCR, kinetic PCR also known as *real time PCR*.

TMA is an extremely sensitive method for detecting HCV RNA. The test involves a three-step process (target capture, amplification, and detection) and has the advantage of being performed in a single tube, thereby eliminating the concern about sample contamination (210). Nucleic acid is released from the sample by a lysis agent and isolated by hybridization to capture oligonucleotides that bind to magnetic microparticles. Amplification of the viral RNA is performed by autocatalytic, isothermal production of RNA transcripts using two enzymes (reverse transcriptase and T7 RNA polymerase) and two primers, one of which contains a T7 promotor. The promotor-containing primer hybridizes to viral RNA, and cDNA is synthesized by reverse transcriptase. The RNA of this complex is degraded by the RNase H activity of the reverse transcriptase,

and a second primer then binds to the cDNA already containing the promotor sequence. Therefore, new DNA can be synthesized by reverse transcriptase. The RNA polymerase recognizes the T7 promotor sequence in the double-stranded DNA molecule and synthesizes numerous RNA transcripts. Each of the

newly synthesized RNAs reenters the TMA process and serves as a template for a new round of replication, resulting in exponential amplification of target RNA. The RNA amplicons are detected by a hybridization protection assay with amplicon-specific chemiluminescent probes and compared to an internal control standard. The sensitivity of TMA is in the range of 25 to 50 copies per milliliter. According to the World Health Organization HCV RNA standard, the sensitivity is 96% (5 IU/mL) and 100% (10 IU/mL) (211).

Quantitative tests for hepatitis C virus ribonucleic acid

Two different technologies have evolved to quantitate HCV RNA levels. These include target amplification methods that use PCR-based technology and signal amplification technologies such as branched DNA (bDNA) assay (212,213,214).

Target amplification methods typically spike the initial reaction mixture with a known amount of a tag that is amplified along with the sample. The ratio of the initial tag to the amount in the final reaction mixture can then be used to estimate the original amount of sample RNA. The major limitations of early PCR tests were sensitivity and the potential for contamination (false-positives). Standardization of commercial tests reduced these problems. Recently, real-time PCR techniques such as TaqMan have been developed. These assays are based on the cleavage of fluorescent dye-labeled probes by the 5′ → 3′ exonuclease activity of the DNA polymerase during PCR. Measurement of fluorescence intensity by a sequence-detection system provides a measure of RNA over time (cycles) and optimizes detection across a wider dynamic range of virus levels. These assays are rapid, are sensitive, have broad dynamic ranges, provide precise quantitation of viral load, and are done in a closed-tube system that prevents crossover contamination by PCR products (215).

Signal amplification is a novel methodology that involves capturing sample nucleic acid in a microtiter well by hybridization with a number of primers targeted to different conserved regions of the genome. An amplification multimer (the branched label probe or bDNA) then hybridizes to the captured RNA complexes, and its signal can then be further amplified and detected by a chemiluminescent reaction. The test is currently marketed by Bayer Diagnostics as the Versant version 3.0 (Table 30.5). The test is highly standardized and reproducible and is accurate over a wide range of viral levels (187,216).

Limitations of hepatitis C virus ribonucleic acid measurement

A major problem and limitation of quantitative HCV RNA testing has always been standardization. This was especially problematic with early tests. A blinded survey of 31 laboratories in Europe demonstrated that only 16% correctly identified all samples in the coded test panel (217). A similar series of laboratory surveys in the United States found accurate identification ranging from 12% to 95% (218). Several factors may have explained these inaccuracies. Delayed serum separation, inadequate storage conditions, and specimen contamination can reduce the amount of nucleic acid in the specimen (219). The sensitivity of the actual assay is limited by the design of amplification primers, length of the amplicon, efficiency of reverse transcription, utilization of reaction substrates and other inefficiencies during amplification, dilution steps, and efficiency of postamplification detection systems (220,221). Even small inefficiencies are exponentially amplified during PCR, and therefore significant errors can be seen. A 12% reduction in efficiency early in the process (e.g., during primer hybridization) results in a 10-fold reduction of the product, and a 24% reduction in efficiency results in a 100-fold decrease (43). Primer hybridization efficiency may also be affected by viral heterogeneity, even within the well-conserved 5′ UTR region used for PCR amplification (87,95), and the use of synthetic RNA transcripts can result in different amplification efficiency for the various HCV genotypes (43). Although this problem potentially affects all PCR-based assay and bDNA systems, appropriate modifications of the reaction mixtures and primers have since corrected the differences in genotype sensitivity in the commercial tests (43,213). Despite the early problems with both qualitative and quantitative HCV RNA assays, testing has been made easier and more reliable by the availability of commercial assays that use familiar test formats that are easily adaptable to hospital laboratories.

Differences in methods lead to variability in quantitation of both the target RNA and standard. Therefore, results with different assays are generally not comparable (222,223,224). Incorporation of a World Health Organization standard into all assays and use of international units were implemented in the hope of resolving these differences, but have not. Therefore, it is important that clinicians understand the technology and limitations of the assays they or their laboratory choose to use. Because levels of HCV RNA measured by different assays are not necessarily the same, care should be taken to ensure that the same assay is used when it

is important to document changes in virus levels (e.g., during treatment).

Hepatitis C Virus Genotyping

HCV is a remarkably heterogeneous family of viruses, with at least six distinct genotypes and numerous subtypes of HCV identified throughout the world (86,87). The methods used to determine genotypes have been described in the preceding text (See "Genotyping"). Currently, the reverse hybridization line probe

assay (LiPA, Bayer Diagnostics, Emeryville, CA) (97) is the most commonly used genotyping method in practice. HCV genotype is highly associated with response to antiviral therapy and is essential in determining the optimal treatment duration (See "Prevention and Treatment").

Histology

The morphologic features of chronic viral hepatitis are similar regardless of the etiology. These features are described in detail in Chapter 26 of this book. In general, the degree of inflammation in chronic hepatitis C is mild to moderate. Severe bridging necrosis and confluent necrosis are unusual in chronic hepatitis C. A few histologic features are highly suggestive of chronic hepatitis C, although they are not entirely pathognomonic. These include epithelial damage of small bile ducts, lymphoid aggregates and sometimes lymphoid follicles in portal tracts, and microvesicular or macrovesicular steatosis (225,226). These histologic features are rarely seen in chronic viral hepatitis B or autoimmune hepatitis and therefore strongly suggest the diagnosis of chronic viral hepatitis C. The pathogenesis of these characteristic changes has not been determined, but the presence of lymphoid aggregates in portal tracts and lymphocyte infiltration of lobules and bile ducts suggest that immune mechanisms play a role in the mediation of cell injury.

Coexistence of chronic viral hepatitis C with other causes of liver diseases is not uncommon and this may modify the morphologic appearance of the liver biopsy. Alcoholic liver disease and chronic viral hepatitis C frequently coexist, and alcoholic liver damage may play an important role in the progression to cirrhosis and hepatocellular carcinoma (HCC) in these patients (227,228,229). Iron overload is more common in patients with chronic viral hepatitis C than in the normal population but does not seem to influence the severity of necroinflammatory activity or fibrosis of the disease (230). Other conditions include coinfection with other viruses, particularly hepatitis B (231,232), and nonalcoholic steatohepatitis. Occasionally, epithelioid granulomas of unknown etiology have been observed in the nodular parenchyma of patients with HCV-related cirrhosis (233).

Table 30.6. Staging Systems for Hepatic Fibrosis Pattern in Patients with Chronic Hepatitis C

Score	Knodell	Ishak	Scheuer	Metavir
0	None	None	None	None
1	Portal	Portal	Portal	Portal
2		Periportal	Periportal	Septae
3	Bridging fibrosis	Focal bridging	Architectural distortion without cirrhosis	Bridging fibrosis
4	Cirrhosis	Diffuse bridging	Cirrhosis	Cirrhosis
5		Extensive bridging		
6		Cirrhosis		

Characterization of the degree of histologic injury is usually performed using one of several scoring systems that utilize descriptive terminology for inflammatory activity and fibrosis in an attempt to group similar degrees of histologic severity. The inflammatory (grade) portions of these different systems typically utilize combinations of numerical assessment for portal, periportal, lobular, and focal inflammation and therefore ignore the specific implications of inflammation in different portions of the lobule. However, inflammation is typically mild to moderate in patients with chronic hepatitis C and is therefore less likely to be important in determining change over time or in making clinical decisions. On the other hand, fibrosis is important in assessing prognosis and has become a critical piece of information in making treatment decisions. Therefore, a quantitative classification of fibrosis (stage) is important. The different staging systems are summarized in Table 30.6. The Knodell system was the first staging system but is noncontiguous and no longer used (234). The Ishak system uses four of its total of seven stages to subdivide the extent of fibrosis between periportal septae and marked bridging (235). This may be helpful in clinical studies when fine gradations of fibrosis pattern are required to

demonstrate change, but this system is not commonly used in practice. The Scheuer and Metavir systems are essentially the same and each has five stages (236,237). Metavir is the most commonly used system in practice today. The accuracy of grading and staging is dependent on the adequacy of

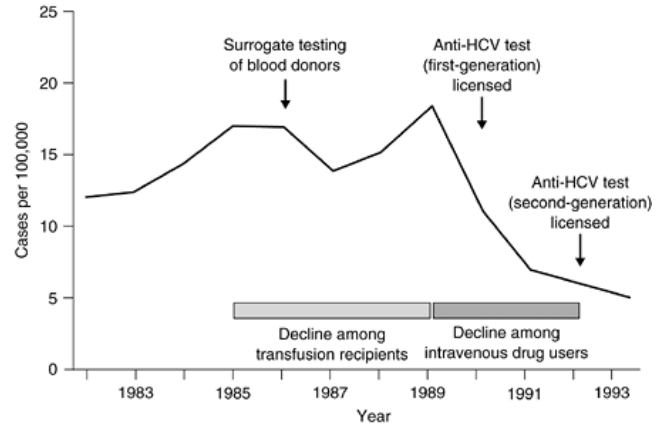
the specimen and the experience of the pathologist, but considerable sampling error can exist as well (238,239).

Epidemiology

Acute Incidence and Population Prevalence

Estimates of the incidence of acute hepatitis C infection in the United States have been extrapolated from age-specific prevalence data and indicate that the new infection rate was low (18/100,000) before 1965, increased rapidly for the next 15 years, and remained high (130/100,000) throughout the 1980s (Fig. 30.6) (240). Prospective surveillance studies conducted by the CDC in four sentinel counties in the United States have indicated a dramatic decline by more than 80% over the last decade (24,241). These rates correspond to 240,000 annual cases in the 1980s and approximately 28,000 to 35,000 cases per year since then (242,243).

The incidence of new infections is most common in young people (aged 20 to 39), especially Hispanics, with a slight predominance of men. The most common risk factor for new infection, accounting for 60% of cases, is intravenous drug use (Fig. 30.7) (243). Identifiable risk factors are present in most acute cases of hepatitis C. Between 10% and 40% of patients do not have a recent or readily identifiable risk factor for infection, although a history of remote high-risk behavior is common in them because such patients tend to underreport such behavior (244,245). Although the incidence of transfusion-associated hepatitis declined dramatically after 1984 because of the testing of potential donors with surrogate markers such as ALT and anti-HBc, and later anti-HCV, this had little impact on the more recent incidence of infection (241). In fact, the recent slowing of new cases is due to a precipitous fall in the occurrence of infections in intravenous drug users (246). This is surprising in the absence of specific public health changes, preventative therapy, or widespread testing for infection.

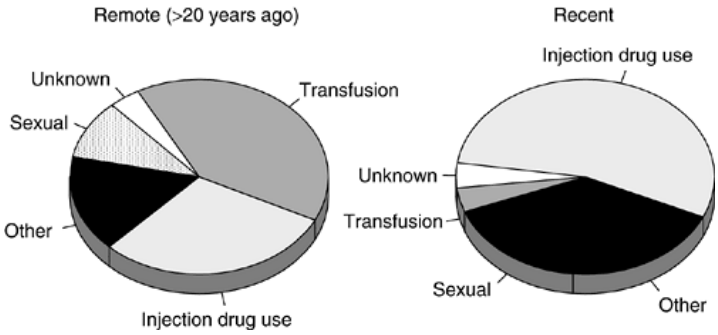


▪ **Figure 30.6** The incidence data in this chart indicate reported cases in the Centers for Disease Control and Prevention Sentinal Countries study of Acute Viral Hepatitis and are not indicative of the national incidence.(See reference 240 for further explanations of incidence calculations.)

The prevalence of HCV infection throughout the world is low, averaging 2% to 3% or 170 million persons (21,247,248,249,250,251). These estimates are often based on volunteer blood donor prevalence rates and may therefore underestimate the true population prevalence (242). Nonetheless, these estimates provide some idea of the worldwide pattern of infection. Overall and age-specific prevalence varies considerably from country to country. Wasley and Alter suggests that this is due to the predominant risk factors (242). For example, in the United States and Europe, the prevalence is low and concentrated in young men who predominantly acquire infection in early adulthood from intravenous drug use (see subsequent text). By contrast, infection is most common in older persons in Japan and Italy, indicating a risk in the distant past. Furthermore, the high prevalence across all adult age-groups in Egypt indicates a common risk factor, namely medical injections (242,252,253). The antibody prevalence is low (0.01% to 0.1%) in the United Kingdom and Scandinavia; slightly higher (0.2% to 1%) in the United States, western Europe, Australia, and parts of South America and Africa; and intermediate (1% to 5%) in eastern Europe, the Mediterranean, Middle East, Indian subcontinent, Brazil, and parts of Africa and Asia (242). The highest prevalence is in northern Africa (6% in Zaire, 7% in Libya, and 17% to 26% in Egypt)

(242,252,253). The

prevalence is also high in some areas of Saudi Arabia (254) and isolated communities in Japan (255).



▪ **Figure 30.7** Sources of acute hepatitis C virus infection over time. (Modified after data from the Centers for Disease Control and Alter MJ. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis* 1997;1:559–568, with permission.)

Although the prevalence of antibodies to HCV is approximately 0.6% in blood donors, the National Health and Nutrition Examination Survey, conducted during 1988 to 1994, found the prevalence of anti-HCV to be 1.8% in the general population of the United States (251,256). Given that approximately 80% of these are probably infected, this accounts for more than 4 million infected persons in the United States. However, some high-risk and high-prevalence populations have not been included in many prevalence surveys. There are currently more than 2 million incarcerated persons in the United States (257), and the prevalence of infection in this group is nearly one in four (258). Therefore, these prevalence estimates may underestimate the true rate. Prevalence begins to rise over the age of 20 and is highest among men aged between 30 and 49 years. African Americans and Hispanics have a higher prevalence of HCV infection than do whites, but the prevalence of HCV infection varies in the population on the basis of risk factors for infection (246). In contrast to what is currently observed in patients presenting with acute hepatitis, transfusion was a more important risk factor in the past (Fig. 30.7). Therefore, transfusion is a common identifiable risk factor only in patients older than 50 years (259).

Chronic hepatitis C is the most common cause of chronic liver disease in the United States, accounting for 40% to 60% of cases (Fig. 30.8) (243,260). This results from the high incidence of acute hepatitis before 1990 and the propensity of acute infection to persist. Because progression to cirrhosis mainly depends on the duration of infection (see subsequent text), each year an estimated 8,000 to 10,000 deaths result from HCV-associated chronic liver disease (243). Most patients who develop cirrhosis have had infection for more than 20 years (261). In the year 2000, approximately 30% of patients with chronic hepatitis C had a history of infection for at least this long (262). This proportion will increase in the future with the cohort of chronically infected patients' age. It is estimated that by the year 2010, more than 60% of patients will have had infection for more than 2 decades (262,263). This has obvious and significant implications for the prevalence of cirrhosis in the infected population. Mathematic models estimate that the proportion of HCV-infected patients with cirrhosis will increase by more than 50% (from 22% of infected cases to 35%) and that complications of cirrhosis such as liver failure and HCC will nearly double (263).

Routes of Transmission

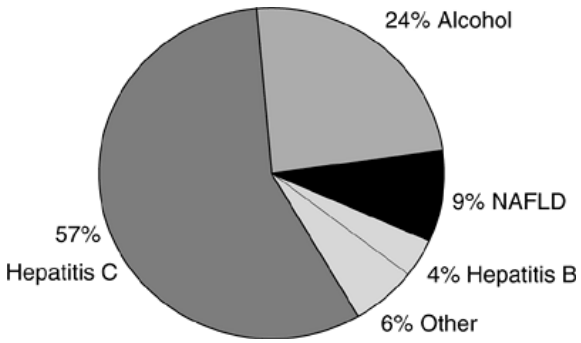
HCV can be transmitted through a variety of routes. Infection is most efficiently transmitted by large or repeated percutaneous exposures such as through transfusions, transplantation from an infected donor, or sharing illicit drug paraphernalia. Transmission of HCV may also occur from exposures to infected contacts through sexual activity, household contacts, perinatal exposure, and parenteral exposures in the health care setting (264).

Intravenous drug use

Intravenous drug use has been the most common risk factor for acquiring HCV infection for more than

25 years and currently accounts for 60% to more than 90% of new infections (Fig. 30.7) (265,266). Sharing of needles and other paraphernalia during parenteral drug use is an extremely efficient means of transmitting infection (243,267). Although both the incidence and prevalence of HCV infection remain high in this group, the incidence of acute hepatitis C among intravenous drug users has declined

dramatically since about 1989 (Fig. 30.6). HCV is rapidly acquired after initiation of drug injection behavior; 50% to 80% of users became anti-HCV positive within 12 months of initiation of drug injection behaviour (268), and nearly all seroconvert by 8 years (269). The risk factors for acquisition of HCV include frequent use, shared paraphernalia, injecting cocaine, sharing with an older user, and long duration of use (270,271). However, it has also been suggested that intranasal cocaine use may be a risk factor (272). Although this route of infection might be possible (e.g., sharing of blood-contaminated straws), it is likely a surrogate for injecting behavior. In addition, two other factors appear to be important in facilitating exposure in this group. Although about a half of infected drug users are either aware of or willing to admit seropositivity, and many continue high-risk behavior despite knowing that they have infection and are at a risk of spreading it to others (244,273).



▪ **Figure 30.8** Etiology of newly diagnosed cases of chronic liver disease. NAFLD, nonalcoholic fatty liver disease. (See reference 260.)

Health care workers; other percutaneous routes

Health care workers have increased exposure to patients infected with HCV. A serologic survey of emergency department patients found that 18% were infected with HCV (274). The proportion with HCV infection was even higher in patients with a history of intravenous drug use (83%), past blood transfusion (21%), or a male homosexual lifestyle (21%) (274). Although all potential routes of transmission of HCV infection to hospital workers are not obvious, needle-stick injuries probably account for a large proportion of cases. Cases of needle-stick transmission of HCV have been clearly documented (275). Skin exposure to blood is not thought to be a risk factor (276). A recent review of follow-up studies of health care workers who sustained percutaneous exposure to blood from anti-HCV–positive patients found that anti-HCV seroconversion after accidental needle-stick/sharp exposures averaged only 1.8% (range, 0% to 6.6%) (Table 30.7) (243,277,278). The risk is greatest with the hollow needles used to draw blood, as compared to hollow infusion needles (278). In another study, an incidence of 10% was found on the basis of detection of HCV RNA by PCR (279). In these prospective studies, none of the infections was associated with mucous membrane or exposure to nonintact skin, although there have been case reports of the transmission of HCV from a blood splash to the conjunctiva (280,281). Several points deserve comments with regard to the risk of transmission by isolated percutaneous exposure. First, the reported risk of transmission by needle stick is greater for HCV than for human immunodeficiency virus (HIV) or HBV (assuming that hepatitis B immunoglobulin is given) (282,283). Second, even a low risk of infectivity has grave implications, given the high risk of progressing to chronic infection.

Table 30.7. Risk of Hepatitis C Virus Transmission after Exposures to Anti–Hepatitis C Virus–Positive Blood

Exposure	Number tested	Number seroconverted (%; range)
Needle sticks/sharps	911	16 (1.8; 0–6.6)
Hollowbore	331	4 (1.2)
Other	105	0

HCV RNA–positive	68	7 (10.3)[a]
Mucous membrane	114	0
Nonintact skin	165	0
Total	1,302	16 (1.2; 0–6.6)

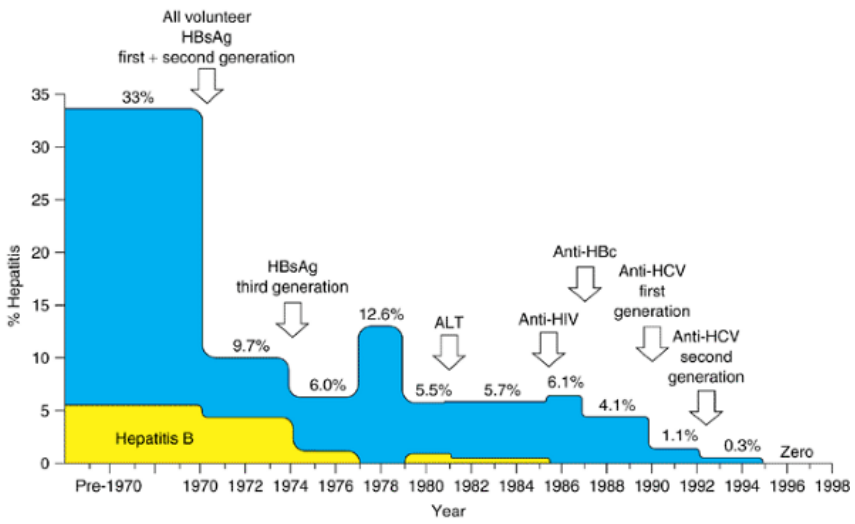
[a]HCV RNA–positive.
HCV, hepatitis C virus; RNA, ribonucleic acid.
See Alter MJ. The epidemiology of acute and chronic hepatitis C. *Clin Liver Dis* 1997;1:559–568.

Although health care workers have increased risk of being exposed to HCV infection, it is debatable whether this occupational exposure results in more than an occasional infection. There were several early reports with seroprevalence rates in health care workers ranging from 0.6% to 4.5%; in all surveys these exceeded the rates in blood donors from the same institution by as much as 4.5-fold (284,285,286). For example, dentists and oral surgeons had seroprevalence rates of 1.75% and 9.3%, respectively, compared with 0.14% in their patients (287). However, other surveys have not found an increased prevalence among health care workers, even in those with regular exposure to blood, including surgeons, dentists, and early responders (276,282,283,284,285,286,287,288).

The risk of nosocomial transmission of blood-borne infections has been dramatically reduced by the use of screening of blood and organ donors, effective

disinfection protocols, disposable equipment, and universal precautions. Transmission of HCV from medical procedures or personnel is exceedingly rare when these precautions are followed. Nonetheless, some cases of nosocomial HCV infection have been reported, although they have usually been associated with breaks in usual technique, such as reusing equipment or multidose drug vials in different patients (See "Hemodialysis") (289). The risk of transmission from an infected health care worker is very low and there does not appear to be sufficient evidence to restrict the practice of these workers. Modeling projections estimate that the risk of transmission from an infected surgeon to a patient is 0.014% or approximately 1:10,000 (290). Nonetheless, there have been two reports of transmission from infected cardiothoracic surgeons. The first, from Spain, involved transmission to five patients over a 6-year period, but the factors responsible for transmission were not identified (291). The other report, from the United Kingdom, found transmission to 1 of 277 at-risk patients during a 1-year period (292).

In countries other than North America and western Europe, unsafe injections, especially involving reusable needles during routine or mass inoculations, have been an important route of nosocomial transmission of HCV. Such practices probably explain the high seroprevalence in Egypt, although these practices appear to have improved (252,253). In many areas, unsafe injection practices, such as reuse of syringes and needles or unnecessary injections, continue (293). Although unsafe injection practices may still be typical in some areas, the lack of available medical supplies is also a factor. Other causes of percutaneous transmission of HCV have included contaminated instruments, equipment, and supplies that were used during the performance of procedures involved in traditional medicine, folk medicine, tattooing, body piercing, and commercial barbering (294,295,296,297,298,299). These routes of transmission have not been documented in the United States.



▪ **Figure 30.9** Risk of post-transfusion hepatitis due to non-A, non-B/hepatitis C in transfusion recipients over the last 3 decades. HbsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; HBc, hepatitis B core; HCV, hepatitis C virus. (See reference Alter HJ, Houghton M. Clinical Medical Research Award. Hepatitis C virus and eliminating post-transfusion hepatitis. *Nat Med* 2000;6:1082–1086, with permission.)

Transfusion associated

HCV is easily transmitted by blood and blood products (22,300,301). In the past, HCV was the major cause of post-transfusion hepatitis, accounting for at least 85% of cases (22,302). As a result, transfusion has often been thought to be the predominant route of transmission of the infection. Early epidemiologic studies supported this because 37% to 58% of patients with chronic hepatitis C, particularly those older than 50 years, gave a history of blood transfusion before 1990 (259). However, the risk of acquiring post-transfusion hepatitis has declined dramatically in recent years (Fig. 30.9) (243,267,303,304,305). The largest drop in post-transfusion hepatitis incidence resulted from adoption of a volunteer donor system and, to a lesser degree, mandatory testing of donor units for hepatitis B surface antigen (HBsAg). Introduction of testing for surrogate

markers of non-A, non-B hepatitis (serum ALT and antibodies to the hepatitis B core [anti-HBc] antigen) reduced the risk of transfusion-associated hepatitis by nearly two thirds (305). Further reduction in the risk of post-transfusion hepatitis was observed after institution of testing for antibodies to HIV and screening of donors for a history of risk factors. Finally, introduction of donor testing for antibodies to HCV was initiated on May 2, 1990, shortly after the discovery of the virus. Retrospective testing of sera from donors and patients who participated in the multicenter Transfusion-Transmitted Viruses Study between 1974 and 1979 predicted that screening of donors for anti-HCV alone would reduce the risk of hepatitis to a level comparable with that of the nontransfused control population (300). Indeed, the incidence of transfusion-associated HCV infection is currently estimated at 0.01% to 0.001% per unit transfused sera (306).

In the past, multiply transfused recipients of blood and blood products had an extremely high risk of acquiring HCV infection. The likelihood of multiply transfused patients developing HCV infection before institution of anti-HCV donor screening was 8.3% in a trauma intensive care unit setting (307) and 18% in a burn unit (308). Patients with transfusion-dependent hemolytic disorders such as thalassemia (309) or hemophilia were at particularly high risk (310,311,312). The risk in patients with hemophilia stemmed from the requirement that factor concentrates be prepared from plasma pooled from hundreds of individuals, who, in many cases, were commercially paid donors (311,312). Before initiation of donor screening, the seroprevalence of anti-HCV ranged from 10% to 16% in paid donors as compared to less than 0.5% in volunteer donors (22,313). Before 1990, between 60% and 90% of factor-dependent patients with hemophilia had serologic evidence of HCV infection (314). HCV infection was more prevalent in those who received greater volumes of concentrate, especially unpasteurized products, and was virtually nonexistent in patients who either had not required factor transfusion or had received exclusively vapor-treated factor concentrates (311). The recent move of vapor-heat sterilization of pooled plasma concentrates and recombinant clotting factors has nearly eliminated the risk of acquiring hepatitis C from replacement therapy (315,316). However, an outbreak of hepatitis C 10 years ago was reportedly associated with contaminated intravenous immunoglobulin (317). This outbreak involved

recipients of a single product produced from the plasma that had been screened by the second-generation anti-HCV assay but on retrospective testing remained positive for HCV RNA (318). Intramuscular immunoglobulin has never been associated with the transmission of any infectious disease in the United States. Currently, all immunoglobulin products (intravenous and intramuscular) commercially available in the United States must undergo an inactivation procedure and be HCV RNA–negative before release.

Hemodialysis

HCV infection is common in patients on dialysis and, currently, is present in approximately 8% of patients in the United States (22,248,319,320,321,322,323). This may, however, be an underestimate because approximately 4% to 15% of infected patients have falsely negative antibodies to HCV (209,321,322). Most dialysis patients are already infected when they present with end-stage renal disease, but acute infection is common in dialysis centers (320,323,324,325). The annual risk of acute HCV infection is currently estimated by the CDC to be 0.15% in hemodialysis patients and 0.03% in continuous ambulatory peritoneal dialysis patients (relative risk, 5.7). Others have estimated the annual incidence to be as high as 0.44% to 1.7% (320). Chronic HCV infection develops in most of those who are acutely infected (70% to 90%) (319). About a third of acute HCV infections are acquired outside the dialysis unit (319,325). However, nosocomial outbreaks have been reported in hemodialysis centers and have been confirmed by molecular sequencing and mapping (326,327). Such infections are usually related to breaks in infection control procedures. Dedicated dialysis machines might reduce much of this risk (328), but this is not common practice in the United States. Other factors such as reuse of multiuse vials and care by specific health care workers have all been implicated (327,329). The cause in many cases remains elusive (329). The improved safety of the blood supply, the availability of recombinant erythropoietin, and the phasing out of pretransplant "immune conditioning" transfusion protocols have significantly reduced the risk of hepatitis C in these patients.

Transplantation

The magnitude of risk assumed by receiving an organ from a donor with HCV infection is subject to some debate. However, HCV infection is not uncommon in cadaveric donors (330,331). In a large study of cadaveric donors evaluated by eight procurement centers in the United States, 4.2% were anti-HCV positive and 2.4% of these were HCV RNA positive by PCR (332). There is no doubt that recipients of organs from these HCV RNA–positive donors are likely to develop infection and liver disease. Some studies have shown nearly universal transmission from anti-HCV–positive donors to recipients (333), whereas others have been unable

to demonstrate such a strong association (331,334). Pereira et al. reported that 75% of the 29 recipients of organs (19 kidneys, 6 hearts, and 4 livers) from 13 anti-HCV–positive donors became anti-HCV or HCV RNA positive (333). By contrast, Roth et al. found post-transplantation liver disease in only 13 of 46 (29%) recipients of kidneys from RIBA-positive donors. HCV RNA levels were not checked (331). Despite the latter study, concern over the risk of transmission is great. Most procurement organizations and transplantation centers have now developed policies for selective utilization of organs from anti-HCV–positive donors (335,336). Modeling strategies have demonstrated that patients without HCV infection who receive an organ from an infected donor incur high cost and have a poor outcome (336). Therefore, donor screening is a necessity. Exclusion of all anti-HCV–positive or HCV RNA–positive donors would incur high costs through the loss of up to 4% of donors and extended waiting times (336). The most cost-effective strategy appears to be transplantation of HCV-positive organs into patients already infected with the virus (336). Most studies have shown that HCV-positive patients who are recipients of an HCV-positive graft have the same graft and recipient survival as those who receive an HCV-negative organ (335,337,338). Furthermore, the waiting time is usually shorter for those who elect to receive an HCV-positive organ (339). After transplantation, either the recipient or the donor strain may become predominant after a few weeks, and this strain then generally persists indefinitely (340). Although the limited data in humans to date does not suggest a genotype advantage in such cases, genotype 1b appears to overtake other genotypes in chimpanzees (341). Despite these many reports to the contrary, however, one recent study has questioned the advisability of using HCV-positive donor organs for kidney transplantation in patients with chronic HCV infection (342).

Sexual transmission

Although sexual transmission is frequently listed in the epidemiology literature as a common risk factor for acquisition of hepatitis C, the data supporting this is poor. Although cases of probable sexual transmission have indeed been reported (343), the extent to which sexual transmission of HCV occurs is not known and it is likely to be exceedingly uncommon during normal sexual activity (264,344,345,346,347,348,349,350,351). The risk of transmission in prospective studies of monogamous heterosexual couples is estimated to be near zero (347,348,349,350). Although these prospective studies have documented a few cases of HCV infection in spouses, these were either phylogenetically unrelated or the spouse had ongoing risk factors in addition to sexual exposure.

The likely explanation for most cases attributed to sexual or so-called sporadic transmission is underreporting of risk factors. One and often many risk factors are present in at least 80% of carefully questioned patients with chronic hepatitis C, but sexual behavior has not been implicated with any certainty as a sole risk factor (352). The risk of overestimating the importance of sexual transmission by not carefully assessing other risk factors has been emphasized (353). Indeed, although the prevalence of infection is usually increased in groups with high-risk sexual practices, it is difficult to exclude all other risk factors for infection. Furthermore, among sexually active individuals without other apparent risk factors attending sexually transmitted disease clinics, the risk does not appear to be increased (344,345).

This being said, there does appear to be circumstantial evidence for sexual transmission among those who practice high-risk sexual behavior. There is a higher seroprevalence of antibodies to HCV, ranging from 0.8% to 22%, in men who have sex with men (MSM) (248,259). In population surveys, antibody prevalence is sixfold higher in MSM than in heterosexuals (259). The risk for HCV appears to be related to the number of sexual contacts, acquisition of other sexually transmitted diseases, use of noninjection drugs during sexual activity, and traumatic sexual practices, particularly anal intercourse (354,355). Heterosexual intercourse, particularly with multiple exposures, is also likely to play a role, although probably a small one, in the transmission of HCV infection. Antibodies to HCV is common in sexual partners of intravenous drug abusers (6%), prostitutes and their clients (3.5% to 9% and 16%, respectively), and heterosexuals with multiple sexual partners (4% to 6%) (343,354). Nonetheless, direct documentation of HCV transmission by high-risk sexual practices has been difficult to obtain. Furthermore, the transmissibility of HCV by this route appears to be extremely low by comparison with HBV, HIV, and other sexually transmitted diseases (354). In one study of sexual partners of HIV–HCV coinfected individuals, 29 pregnancies, one HIV seroconversion, and no cases of HCV infection occurred during an estimated 5,800 unprotected vaginal or anal contacts (351). Importantly and not to be forgotten, barrier contraception with condoms has been shown to reduce the risk of transmission of sexually transmitted infections.

Perinatal transmission

Perinatal transmission is known to occur, but the predominant route (i.e., intrauterine, intrapartum, or perinatal) is not known (356). Antibodies to HCV usually passively transferred from the infected mother to the infant and may remain detectable in the baby for

up to a year (357,358). The risk of transmission of HCV from viremic (HCV RNA–positive) mothers to their infants is 3.2% (356,359). The risk of transmission in viremic women coinfected with HCV and HIV (HCV RNA positive) is 7.9% (359). This higher rate of transmission may, in part, relate to higher levels of HCV RNA in coinfected women; however, some studies have not shown a relationship between risk and viral levels (356,358,360,361). The rate of mother-to-infant transmission is similar for vaginal and caesarean delivery (356). However, prolonged duration of membrane rupture appeared to increase the risk of infection in the infant in one study (362). HCV is not transmitted by breast-feeding (363). Finally, despite development of infection (HCV RNA positive) in the infant, occurrence of abnormal serum ALT levels or liver disease is unusual, with a weighted rate of 1.7% (356,357,358,359).

Sporadic hepatitis

Large public health surveys have suggested that up to 40% of patients with acute hepatitis C have no identifiable risk factor (24,241,267,284). These cases have been called *sporadic* or *community-acquired hepatitis*. The implication is that such cases result from previously unidentified modes of transmission. However, the fact that nearly all anti-HCV–positive patients with chronic liver disease have identifiable risk factors suggests the possibility that the number of sporadic cases is not as great as that reported, and the lack of identifiable risk factors may be more dependent on the patients' recognition of or willingness to reveal their high-risk behaviors (353,364).

The prevalence of HCV infection in the population and the difficulties in clarifying its epidemiology make it obvious that a search for other routes of transmission is important. Although some studies have failed to identify nonparenteral routes of transmission (346), familial and community clustering of hepatitis C cases with seroprevalence of antibodies to HCV ranging from 0% to 34% have been reported (365,366). Although sexual partners appear to be at increased risk in such surveys (366,367), a similar antibody prevalence has been noted in nonsexually exposed family members including children, parents, and siblings, particularly in the older individuals exposed for a longer period (367,368). Sharing of hygiene items such as combs, razors, toothbrushes, and nail scissors has been proposed as a possible means of transmission in such cases (369). Saliva has been implicated as a vector of transmission (370), and indeed, infection through saliva has been documented in chimpanzees (371) and by a human bite (372). However, the existence of infectious virus in saliva is controversial (370,373,374). Occasional casual contact is not likely to transmit infection, and only 3% of patients with acute HCV infection give such a history (284).

Natural History

Acute Hepatitis C Virus Infection

Clinical presentation

Acute hepatitis C is typically asymptomatic and unrecognized. When identified prospectively, cases present with an elevation of the levels of serum aminotransferases anywhere from 2 to 26 weeks after exposure (375). The mean incubation period is intermediate between that for hepatitis A and hepatitis B, with a peak onset of 7 to 8 weeks after infection. Eighty percent of cases occur between 5 and 12 weeks (376). It is not known whether the route of infection, inoculum size, viral genotype, or other factors influence the variability in the incubation period. However, a shorter incubation period was noted in several early reports of hepatitis after transfusion of pooled blood products (377,378). Serum ALT levels are usually high, with about three fourths of patients having elevations more than 15 times the upper limit of normal (379). HCV RNA is present in the blood within days of exposure and usually remains detectable throughout the infection (380). By contrast, anti-HCV is often not detectable until 5 to 6 weeks after exposure (204,206). The mean times to detection of anti-HCV by the second- and third-generation tests are 10 weeks and 7 to 8 weeks, respectively (204,206). Symptoms occur in less than 30% of patients and are usually so mild that they do not interfere with the daily routine (24). When present, the symptoms of acute hepatitis C are nonspecific and do not differ from other forms of hepatitis. The most common symptoms are flu-like and include anorexia, weight loss, abdominal pain, myalgia, arthralgia, and fatigue. Less common symptoms include fever and rash. Jaundice occurs in less than one third of all patients (24,379) and is most common in symptomatic patients (375). The symptoms associated with acute hepatitis usually resolve within 1 to 3 months.

Fulminant hepatic failure due to HCV is extremely uncommon (381). Although some early studies reported detection of serum HCV RNA in up to 60% (mean, approximately 10%) of fulminant hepatitis cases without an obvious etiology (382,383,384,385,386), most clinicians feel that HCV accounts for only rare cases. Large studies in the United States and Japan have failed to identify any case (387,388).

Risk of chronicity

Early reports based on observations in transfusion recipients suggested that about half of the patients with

acute non-A, non-B hepatitis recovered spontaneously; later reports challenged that this event occurred in only 10% to 20% of cases (376,389,390). It is clear that there is considerable variability in the risk of progression to chronic infection, with a range from 50% to 90% (390,391). Resolution of acute infection appears to be more common in young people and women but may also be related to viral genotype (391). Clearance of acute infection is documented by persistent loss of detectable virus because liver test results may normalize or the virus may become transiently undetectable in some patients who go on to develop chronic infection (392). Indeed, chronic viremia persists in at least half of the 30% to 40% of cases in whom the serum ALT level returns to normal (375). Overall, approximately 80% of acutely infected patients develop chronic infection (viremia) and most of these (80% to 90%) have chronic hepatitis with elevated ALT levels (248,393,394,395,396,397,398,399). Factors that determine viral clearance or persistence are not clear. Although several retrospective serologic studies have identified anti-HCV–positive patients who are HCV RNA negative, suggesting the possibility of late viral clearance, it is not possible to exclude the possibility that in these cases virus clearance occurred during acute infection (396,400,401). Indeed, most data suggest that spontaneous eradication of chronic infection is extremely unusual (402).

Chronic Hepatitis C Virus Infection

Clinical presentation

Most patients with chronic hepatitis have asymptomatic elevations of serum aminotransferase levels and do not have physical signs of liver disease (403). Only about 6% have symptomatic liver disease (404). Fatigue is the most common symptom, but its onset is insidious and is usually mild (403). Dull right upper quadrant pain, which is often intermittent and positional, is also common. Less common symptoms include anorexia, nausea, pruritus, arthralgia, and myalgia. Importantly, although symptoms are more common in patients with fibrosis or cirrhosis, the correlation with the histologic severity of disease in individual patients is poor (403). The physical examination may be more helpful once cirrhosis has developed. In these patients, a palpable firm liver is present in 79%, splenomegaly in 34%, and stigmata of chronic liver disease in 31% (405).

Serum ALT levels are usually only mildly elevated. Up to one third of patients have normal serum ALT levels, whereas only about 25% have a level more than twice normal (261,405,406). However, there is a wide variability in enzyme level elevation when patients are followed up over time. In those with ALT level elevations, the levels are persistently elevated in only 26% of cases and elevated in most

determinations in 22%. ALT levels fluctuate down to the normal range in 17%. Finally, 30% to 40% of cases have only occasional ALT level elevations over a 12- to 18-month period of testing (375,376). Furthermore, although in group analyses higher ALT levels suggest more hepatic inflammation, the variability is marked and ALT has little predictive value in individual patients (261,403,407,408). Only a 10-fold or greater elevation of serum ALT level is predictably associated with significant piecemeal necrosis (408). Therefore, ALT alone should not be used to estimate disease severity, prognosis, or necessity of treatment.

Histologic and clinical progression

Although chronic hepatitis C develops in approximately 80% of acutely infected individuals, the disease progresses slowly, if at all, in most patients. However, the true rate of histologic progression has been the subject of debate and uncertainty. Some authorities have suggested that progression to severe end-stage liver disease is inevitable provided the infected person does not succumb first to another lethal illness, whereas others have concluded that disease progression is extremely unusual and restricted to a limited few. These opposing views can be accounted for by the slow pace of progression, the usual lack of symptoms in chronically infected patients, and the limitations of available natural history data (390). For example, studies based on exposed or acutely infected patients such as the post-transfusion hepatitis studies of the 1970s report few significant sequelae of infection (see subsequent text), whereas short-term prospective and retrospective studies of patients who already have established liver disease clearly show a significant risk of progressing to cirrhosis, liver failure, and HCC (409,410,411). The limitations of each of these types of studies have been discussed (411). Interestingly, mathematic models of the natural history of chronic hepatitis C appears to resolve this debate (412). These clearly demonstrate that both perceptions of the natural history are accurate, but progression is more easily observed in studies focused on the later stages of the disease.

Several long-term follow-up studies of recipients of contaminated blood products show that few patients with acute hepatitis C progress to liver failure and liver-related death. The classic study by Seeff reported the outcome in patients who had participated in five transfusion surveillance studies from 1968 to 1980 and had been prospectively followed up after the development of acute transfusion-associated non-A, non-B hepatitis (mostly hepatitis C) (404,413,414,415,416,417). Approximately half of these individuals were known to have developed chronic hepatitis with elevated aminotransferase levels, and, of these, slightly more than 30% had developed cirrhosis (411). Although more than 40% of patients with cirrhosis had some

evidence of hepatic decompensation, all-cause mortality was not significantly different from that of transfusion recipients who had not developed hepatitis (404). Most deaths were due to cardiac disease, which was not unexpected because most subjects received their transfusions during cardiac surgery. Nevertheless, liver-related mortality after 18 years was higher among patients with hepatitis (3.2%) than in controls (1.5%). A follow-up report 7 years later found a liver-related mortality of 4.1% versus 1.3% in controls (418). In both groups, liver-related mortality was strongly correlated with alcohol consumption. A similar study among HIV-negative, transfusion-dependent patients with hereditary bleeding disorders found liver-related mortality of just 3.4% after 25 years (396). A major limitation of transfusion studies is the high (50% to 67%) all-cause mortality related to the comorbid conditions that necessitated transfusion in the first place (404,418,419). However, long-term retrospective data are available in healthy young women who received HCV-contaminated lots of immunoglobulin products more than 20 years ago (420,421,422). More than 400 of the 53,178 (0.8%) recipients of eight HCV-contaminated lots of anti-D immunoglobulin in Ireland in 1977 were found to be anti-HCV positive on subsequent testing 17 years later (420). Liver biopsies revealed mild or moderate hepatitis in 93%. Although 15% already had bridging fibrosis, only 2% had cirrhosis. In a similar study from Germany, 160 of 2,533 (6.3%) women who received anti-D immunoglobulin between 1978 and 1979 became anti-HCV positive (421). Slightly more than half developed chronic hepatitis, but a large proportion appeared to recover completely. The low transmission and high recovery rates in these studies are quite atypical and may relate to the size of the inoculum, age of recipients, or both. Nonetheless, taken together, the studies in transfusion and immunoglobulin recipients confirm that most patients with acute hepatitis infection do well and are not at high risk of developing cirrhosis or liver failure, even after 20 to 25 years.

In contrast to these studies, some prospective studies of patients with acute post-transfusion hepatitis C have reported cirrhosis in 16% to 24% after follow-up of just 8 to 14 years (262,409,423,424,425). The prevalence of cirrhosis may increase to more than 40% after 40 years (426). Furthermore, the subset of patients who present with established chronic hepatitis C appears to have an even higher rate of progression to cirrhosis, liver failure, and liver-related death, although a self-selection bias is probably responsible for this observation (261,427,428,429). Evidence to support this derives from the estimated duration of infection in patients presenting with complications of liver disease; it is similar to the period of observation in the transfusion studies described earlier. Retrospective studies of patients with cirrhosis have suggested that the mean duration of infection before the development of cirrhosis is about 21 years, although this interval may occasionally exceed 50 years (261,430).

The rate of progression to cirrhosis has been estimated in several studies but is probably not linear in most individuals and depends upon several host and environmental factors (see subsequent text). The annual rate of fibrosis progression is estimated by dividing the fibrosis score (Metavir stage) by the estimated number of years after infection (431,432). Using this method, the average rate of fibrosis accumulation is 0.133 units per year (95% CI, 0.125 to 0.143) in patients with elevated serum ALT levels (432,433). Therefore, if progression is linear, it will take an average of 30 years to develop cirrhosis. However, many factors may influence the rate of progression in individuals, and therefore, it has been proposed that most patients fall into one of three groups with fibrosis rates that are rapid (less than 20 years to cirrhosis), intermediate (late cirrhosis), or slow (no cirrhosis or after 50 years) (433). Patients with persistently normal liver test results fall into the latter group because their mean fibrosis rate is only 0.05 units per year, which extrapolates to a mean time to cirrhosis of 80 years (434). Hepatic inflammation also influences the rate of progression (428,433). The 10-year risk of cirrhosis is less than 10% to 13% among patients with minimal or mild chronic hepatitis and 44% to 100% in those with moderate hepatitis (Table 30.8) (428,433).

There are several environmental, host, and viral cofactors that accelerate disease progression. The most important of these cofactors is alcohol intake (227,435). The association between HCV infection and alcoholic liver disease was first noted in early epidemiologic surveys of anti-HCV prevalence (435,436,437). It is now apparent that regular alcohol intake, particularly heavy intake, may accelerate liver injury in persons with chronic HCV infection (435,436,437,438,439,440,441). The risk of progressing to cirrhosis appears to be 1.5 to 3 times higher in those who consume alcohol (435,438,439,442,443,444,445). Furthermore, the effect appears to be dose–dependent, with a 15-fold higher

risk in the heaviest consumer compared to teetotalers (446). Alcohol presents greater risk for progression than duration of infection, age, gender, or coinfection with either HBV or HIV (444). Finally, patients who die from liver disease are more likely than others to be alcohol users (447). It is not yet clear whether the mechanism for this outcome is increased HCV replication or an additive injury from both the virus and the alcohol (228,435,448). Heavy alcohol use has been shown to inhibit hepatic expression of Bcl-2, an inhibitor of apoptosis, and to increase oxidant exposure in patients with chronic hepatitis C (449,450). Regardless of the mechanism, the outcome of alcohol use is clear and its use in persons with chronic HCV infection is to be strongly condemned. Evidence supporting a role for other environmental factors, such as toxins, in the progression of HCV infection is limited.

Table 30.8. Likelihood of Histologic Progression to Cirrhosis

Initial histology	Risk of cirrhosis (%)		
	5 y	10 y	20 y
Minimal-to-mild hepatitis	7	7	30
Moderate hepatitis	25	44	95
Severe hepatitis	68	100	100
Bridging fibrosis	58	100	100

Adapted from Yano M, Kumada H, Kage M, et al. The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996;23:1334–1340.

Other host-related factors are also important. The rate of disease progression seems to increase if HCV infection is acquired after the age of 50 to 55 years (261,428,432). Age may also play a role in those already infected chronically and might explain the more rapid progression that sometimes occurs as the disease advances beyond the second decade (428,432). The low rate of both acute and chronic infection in the contaminated anti-D immunoglobulin studies suggests the possibility that female gender, possibly in combination with young age, might reduce the risk of infection (420,421). Other host-related factors, particularly ethnic background, deserve further study because there is some evidence that disease progression and the development of HCC is more common in Japan and Italy (451). African Americans appear to have histologically somewhat less severe liver disease than do Hispanics or whites (452). Finally, host immune status may influence progression. Some studies suggest that HLA DRB1 might be important in the susceptibility to infection (453), and other phenotypes might be important in defining

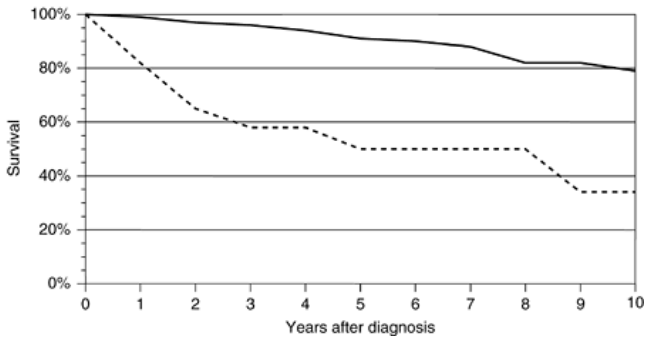
the host's ability to regulate viral replication (454). Furthermore, immunosuppression from HIV infection or immunosuppressive drugs after organ transplantation may accelerate disease. HIV-infected patients have higher HCV levels (455) and a higher rate of fibrosis progression, although there may be other confounding factors involved (400,456). Progression to cirrhosis is threefold higher in HIV-infected persons and is more common in patients with low CD4 counts (457). Liver transplantation and the necessity for exogenous immunosuppression results in accelerated liver injury. HCV infection persists in almost all patients who undergo transplantation for chronic hepatitis C and results in severe and progressive chronic hepatitis in many (458,459). Virus levels increase 10- to 15-fold after transplantation (459). The actuarial risk of developing cirrhosis is 3.7%, 8.5%, and 28% after just 1, 2, and 5 years of transplantation, respectively (460). More than 40% of these patients with cirrhosis will decompensate within 1 year, and survival is only 41% within a year of the decompensation event (461). Surprisingly, however, rapid progression and decreased early survival is not present in recipients of other solid organs or bone marrow (462,463,464).

There is currently insufficient data to suggest that viral factors influence the progression of HCV infection. Early studies suggested that genotype 1 caused more severe disease than genotype 2 (465) and that 1b was more harmful than 1a (466,467). However, the relationship of genotype 1 to more severe disease may be related to a longer duration of infection, and more recent studies suggest that genotype plays no role in influencing disease outcome (468,469). However, viral genotype significant influences treatment response and may therefore have considerable impact on the long-term disease outcome (see subsequent text).

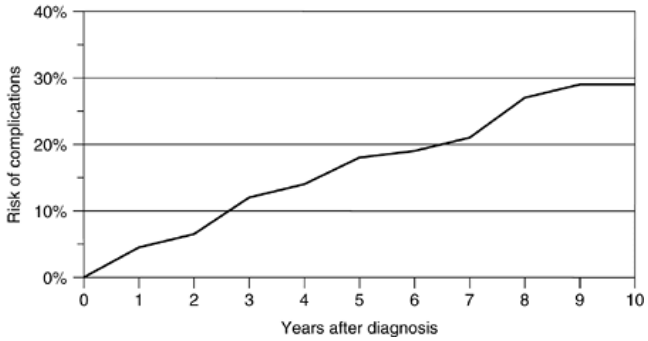
Liver-related morbidity and mortality among HCV-infected persons is directly related to complications of cirrhosis including hepatic failure and HCC (405,428,429). Liver-related mortality in those with compensated cirrhosis is low before the first episode of decompensation supervenes or the onset of synthetic dysfunction (Fig. 30.10) (405,470). Among patients with compensated cirrhosis, the annual risk of decompensation is 3.9% (Fig. 30.11). Decompensation is most commonly manifested by the development of ascites (48%) or variceal hemorrhage (22%), although multiple complications may occur simultaneously (17%) (405,470). At least one third of deaths in patients with cirrhosis occur as a consequence of these two complications. When ascites first presents, it can usually be managed quite easily with diuretics and salt restriction. Varices can often be managed effectively with endoscopic therapy. However, one should not be lulled into complacency with patients who can be managed medically because the excess mortality is still 10% to 33% after 3 years (471). Therefore, such patients should be considered for transplantation at the time when ascites or bleeding varices first appear. Survival in patients with decompensated cirrhosis is poor, with only approximately 50% surviving for more than 5 years (Fig. 30.10) (405). Hepatic synthetic dysfunction alone without other complications of cirrhosis has less impact on survival. Elevated bilirubin level, decreased albumin level, or thrombocytopenia results in a 16% to 19% decrement in 10-year survival compared with those with normal synthetic function (405). However, an elevated prothrombin time is a poor prognostic sign associated with a 39% reduction in 10-year survival compared with patients with cirrhosis who have normal synthetic function (405).

HCC is the fifth most common cancer worldwide. HCC is a significant complication of HCV infection and HCV is the most common etiologic factor in the United

States (472). Its incidence is also increasing in Europe (473). HCV-related HCC rarely occurs in the absence of cirrhosis (474,475). The annual risk of HCC is 1.4% to 3.3% in patients with cirrhosis in the United States, most of Europe, and Australia (261,405,476,477,478). It is estimated that the risk of HCC will double over the next 10 years (263) and may approach the higher risk rates (2.6% to 6.9%) observed in Japan and Italy (439). The mechanism for development of HCC in HCV disease is not known. Associations with small cell dysplasia and mutations in the protein kinase receptor binding domain, serine protease region, or CD81 genes have been reported (479,480,481). There is no association with mutations of tumor suppressor genes, virus levels, or viral genotype (482,483). The mean duration of HCV infection in patients with HCC is 28 to 29 years (261,405). HCC is more common in the presence of the following factors: Longer disease duration, hepatic synthetic dysfunction, cytopenia, male gender, and alcohol use (438,476,477,478,479,480,481,482,483,484,485). HCC appears to occur earlier in HIV-coinfected patients (486). Diagnosis of HCC is by imaging examinations and α-fetoprotein (AFP). AFP level is elevated in a variable proportion of patients with HCC and the risk of HCC is increased 30-fold when the AFP level is elevated (485,487). However, AFP is not specific and its level may be elevated in 30% to 45% of patients with chronic hepatitis C without HCC, although it is usually less than 100 ng/mL (405,487,488,489,490). Magnetic resonance is the most sensitive screening test, followed by computerized tomography and ultrasonography (490). However, the more sensitive imaging modalities are quite expensive. Imaging is most sensitive when performed every 6 months (490). Treatment of HCC is discussed in Chapter 44 of this book.



▪ **Figure 30.10** Survival in patients with cirrhosis due to chronic hepatitis C. Effect of complications and decompensation (*top line*, compensated cirrhosis; *bottom line*, decompensated cirrhosis). (See Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472, with permission.)



▪ **Figure 30.11** Risk of developing decompensated liver disease among patients with stable cirrhosis due to chronic hepatitis C. (See Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472, with permission.)

Currently, hepatic decompensation and HCC related to chronic hepatitis C are common indications for liver transplantation. HCV is the leading indication for liver

transplantation in the United States, accounting for approximately half of the nearly 6,000 liver transplantations performed each year. Liver transplantation for hepatitis C is described in detail in Chapter 53 of this book.

Prevention and Treatment

Public Health, Passive Protection, and Vaccines

Public health

Health care professionals must be informed about appropriate medical management of HCV-infected patients, known and potential risks for infection, the need to identify risk factors in their patients, appropriate evaluation of high-risk patients, and recommendations for prevention. All anti-HCV–positive patients should be considered infectious and informed of the possibility of transmission to others, although no tests are available to reliably determine infectivity. Counseling recommendations to prevent transmission of HCV were published by the U.S. Public Health Service in 1998 and were incorporated, in part, into the 2002 National Institutes of Health (NIH) Consensus Development Conference report on the management of hepatitis C (491,492). These recommendations are summarized in Table 30.9.

Passive protection

Neutralizing antibodies directed against immunodominant epitopes of HCV should protect the susceptible hepatocytes and other target cells from HCV infection. Experimental studies in chimpanzees have shown that plasma from a patient with chronic hepatitis C was capable of neutralizing isolate-specific HCV in vitro, thereby preventing infection (123). Neutralizing antibodies may be acquired passively by the administration of immunoglobulin preparations containing these antibodies. Before identification of HCV and elimination of anti-HCV–positive patients from the donor pool, it would be reasonable to assume that antibodies directed against HCV envelope proteins were present in the immunoglobulin prepared from pooled donors. On the basis of this assumption, passive immunization with conventionally prepared immunoglobulin was attempted to reduce the risk of transfusion-associated non-A, non-B hepatitis. Four prospective, randomized (three of which were placebo-controlled) clinical trials of immunoglobulin for the prevention of transfusion-associated non-A, non-B hepatitis provided conflicting results (415,417,493,494). Piazza et al. found that only 1 of 450 heterosexual partners of patients with HCV antibodies who received immunoglobulin enriched with anti-HCV became infected compared to 6 of 449 partners who received placebo (495). Feray et al. demonstrated indirect evidence that passive immunization may prevent HCV infection in liver transplant recipients. Among 218 HBsAg-positive patients who had HCV coinfection before transplantation, the prevalence of HCV viremia after transplantation was lower in those receiving hepatitis B immunoglobulin prepared before 1990 when screening of donors for anti-HCV began (25 of 46 [56%] patients) compared to others who received immunoglobulin free of HCV antibody (162 of 172 [94%] patients) (496). The 3-year actuarial rate of recurrent hepatitis C was also lower in those who underwent transplantation before 1990 (10%) than in those who underwent transplantation after 1990 (61%). Among patients who did not receive immunoglobulin therapy, the proportion who developed hepatitis C after transplantation was similar to that in patients who underwent transplantation before (63%) or after 1990 (71%) (496).

These studies suggested the possibility that pretransfusion administration of immunoglobulin containing antibodies to HCV would reduce the risk of non-A, non-B hepatitis, but the results are far from conclusive. Furthermore, these studies have much less relevance today. First, plasma collected for fractionation is screened for anti-HCV, and it is therefore likely that HCV-neutralizing antibodies are either absent or present in exceedingly low titers in currently manufactured conventional immunoglobulin. Second, the risk of post-transfusion hepatitis has nearly been eliminated by donor screening (306). In summary, postexposure prophylaxis with standard immunoglobulin is not effective in preventing HCV infection and is not recommended by the Advisory Committee on Immunization Practices (277).

It might be possible to produce immunoglobulin containing high titers of HCV-neutralizing antibodies. In fact, hyperimmune serum prepared against a synthetic peptide derived from the HVR of the HCV envelope protein is capable of neutralizing HCV and preventing infection when mixed with HCV in vitro before inoculation into chimpanzees (497). Postexposure prevention of HCV infection has also been studied by using a human HCV hyperimmunoglobulin prepared from anti-HCV–positive plasma that tested negative for HCV RNA (498). Intravenous infusion of this polyclonal product into a chimpanzee within 1 hour of inoculation with an infectious dose of HCV did not prevent infection but did appear to delay the onset of liver injury. Similar preparations have now been used in humans (499,500). Both studies infused antibody at the time of and after liver transplantation in patients with chronic hepatitis C. Neither study demonstrated any effect on HCV RNA levels or recurrence in the graft.

Table 30.9. Guidelines for High-Risk and Hepatitis C Virus–Infected Individuals from the 2002 National Institutes of Health Consensus Development Conference on Management of Hepatitis C (491,492)

HIGH-RISK BEHAVIOR

- Get vaccinated against hepatitis A and B
- Persons who continue to use or inject illegal drugs should
  Never reuse or share syringes, needles, water, or drug
  Use only new and sterile syringes and water obtained from a reliable source
  Use new or disinfected containers and filters to prepare drugs
  Clean the injection site with alcohol
  Dispose of syringes after one use
- Persons at risk for sexually transmitted diseases should
  Have sex only with a single uninfected partner or abstain, which is the best way to avoid infection
  If having high-risk sexual activity, use latex condoms correctly and every time

HEMODIALYSIS PATIENTS

- Use dedicated dialysis stations, chairs, and beds; clean after each use
- Avoid sharing ancillary nondisposable supplies; clean after each use
- Medication and supplies should not be shared and medication carts should not be used
- A central medication and supply prep area should be used
- Separate clean and contaminated areas

HCV-INFECTED PERSONS

- Consider all anti-HCV or HCV RNA–positive persons to be potentially infectious
- Do not donate blood, organs, tissues, or semen
- Avoid sharing of household items such as toothbrushes and razors
- Changes in sexual practices are not required within a monogamous relationship (however, a low potential for sexual HCV transmission exists; anti-HCV–positive persons and their partners should be informed of the potential risk of sexual transmission and an informed decision regarding the need for precautions should be made)
- Vaccinate against hepatitis A and B
- Avoid exposure to risk factors for transmission to others
- Avoid alcohol consumption

PERSONS REQUIRING ROUTINE TESTING FOR HCV INFECTION

- Persons who have at any time injected illegal drugs regardless of number of events
- Persons who received clotting factor concentrates before 1987
- Persons who were at any time on long-term hemodialysis
- Persons with persistently abnormal alanine aminotransferase levels
- Persons who received a transfusion or organ transplant July 1992
- Persons with needlestick, sharp, or splash exposure to HCV-positive blood
- Children born to an HCV-positive mother
- Routine testing is *not* recommended for:
  Health care, emergency medical or public safety workers
  Pregnant women
  Household contacts
  General population

HCV, hepatitis C virus; RNA, ribonucleic acid.

Because of the lack of an effective immunoglobulin, persons with percutaneous exposure to HCV should be closely monitored for infection. At a minimum, this requires serial testing of serum ALT and anti-HCV levels at baseline and 4 to 6 months postexposure (491). However, others have suggested serial HCV RNA determinations by PCR to identify early acute infection that might prove more amenable to IFN treatment (see subsequent text) (501).

Vaccination

Development of an effective vaccine against HCV has faced several obstacles. First, it appears that neutralizing antibodies against the hypervariable envelope proteins, although potentially protective, are largely isolate specific and therefore likely to provide only temporary protection against a heterogeneous virus such as HCV (126). Second, little is yet known about the ability of HCV-specific cellular immune responses to induce protection. Finally, it will be difficult to test the efficacy of a potentially protective vaccine when the risk of acute infection is so low. Nonetheless, some progress has been made in developing hepatitis C vaccines. A recombinant E1E2 heterodimer vaccine administered four times over a year to healthy volunteers induced antibodies that bound to E1E2 and

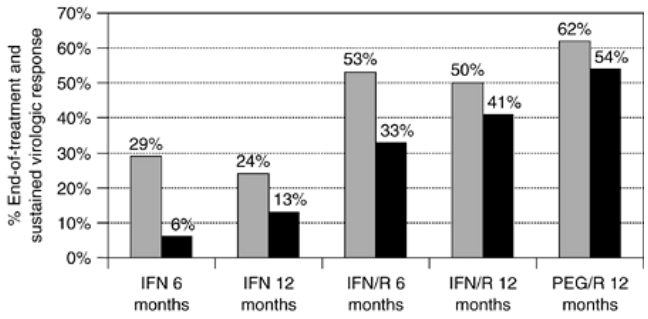
CD81, the putative HCV receptor (502). Furthermore, the antibodies neutralized E1E2-encoated vesicular stomatitis virus pseudovirions and induced lymphocyte proliferation. In another study, a recombinant E1 vaccine was given to 24 HCV-infected patients in repeated courses. All but three patients developed a significant de novo E1-specific T-cell response. Anti-E1 antibodies increased and higher levels correlated with the decrease in total Ishak score in 9 of the 24 subjects (503).

Antiviral Therapy

The major goal of treatment of HCV infection is to prevent the development of decompensated liver disease and death. This can be accomplished by preventing new infections, reducing the chance of acute infection progressing to chronic hepatitis, or effectively treating chronic infection. The goals in treating chronic hepatitis should include eradication or prolonged suppression of viral replication, reduction of hepatic inflammation, and ultimately, slowing the rate of progressive liver injury. Not all these goals may be achievable in every patient. However, eradication of chronic HCV infection is now possible in half or more of treated patients (504).

Evolution of treatment for chronic hepatitis C

Increasingly more effective treatment regimens for chronic hepatitis C have evolved rapidly over the last 2 decades (Fig. 30.12). In the mid-1980s, IFNs became the first agent studied for the treatment of what was then called *chronic non-A, non-B hepatitis*. IFNs are glycoproteins produced in vivo by leukocytes in response to viral infection. IFNs can be commercially manufactured by cell culture or recombinant technology and have been commercially available for the treatment of chronic hepatitis for more than a decade. IFNs inhibit the replication of many viruses, including hepatitis viruses, through a variety of mechanisms including direct antiviral mechanisms (inhibition of virus attachment and uncoating, and induction of intracellular proteins and ribonucleases) and amplification of specific (CTL) and nonspecific (NK cell) immune responses (505). The specific mechanism(s) of action for IFN in chronic hepatitis C infection remain(s) poorly understood. However, viral kinetic modeling in IFN-treated subjects shows a biphasic decline in HCV RNA level after IFN treatment (506). The first 24- to 48-hour phase is characterized by a rapid decline in virus level, which is thought to represent degradation of free virus, while replication of new virions and infection of naïve cells is inhibited (506). The second phase has a slow exponential decline in viral levels and is thought to represent loss of residual infected hepatocytes (506).



▪ **Figure 30.12** Changes in the end-of-treatment and sustained response rates with different interferon (IFN) treatment regimens (*shaded bar*, end-of-treatment response; *solid bar*, sustained response). IFN/R, interferon and ribavirin; PEG/R, polyethylene glycol and ribavirin.

Recombinant IFN was first approved by the U.S. Food and Drug Administration (FDA) for the treatment of non-A, non-B hepatitis in 1991. Initially, the recommended treatment course was 6 months, but it was subsequently shown that prolonging treatment to 12 months doubled the sustained response rate, and this longer regimen was subsequently approved as the standard of care (507,508,509). Sustained biochemical (normalization of the serum ALT level) and virologic (loss of detectable serum HCV RNA) responses were relatively uncommon with IFN monotherapy, ranging from 6% to 15% after a 6-month course of IFN to 13% to 25% after 12 months of therapy (509). More recent studies that have carefully assessed virologic endpoints of treatment with different regimens have clearly shown that sustained responses to IFN monotherapy are at the lower end of these previously described ranges (510,511).

Ribavirin was added to the treatment regimen almost 10 years later. Ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside analog that structurally resembles guanosine (512). Ribavirin has in vitro activity against several DNA and RNA viruses, including Flaviviridae (513). The mechanism of action in HCV infection is not clear, but the

predominant current opinion is that ribavirin induces lethal mutations in viral genome, a mechanism known as *viral error catastrophe* (512,513,514). Early studies of ribavirin alone found that serum ALT levels fell to within the normal range in 40% of treated patients, but virus levels did not significantly change (515,516,517). However, when combined with IFN, ribavirin reduces relapse after response

during treatment, and this results in a dramatic improvement in the sustained virologic response (SVR) rate (510,511,518,519). The results of two similarly designed large randomized controlled trials comparing combination therapy to IFN monotherapy have been combined and reported (520). These studies compared 6- and 12-month courses of combination therapy (3 million units of IFN-α-2b thrice weekly plus 1,000 to 1,200 mg oral ribavirin daily) to similar duration courses of IFN alone (510,511). The combined results showed sustained viral-negative responses in 41% and 33% of subjects treated with 12 and 6 months of combination therapy, respectively, compared to 16% and 6% in those treated with IFN alone for 12 and 6 months, respectively (520).

Long-acting pegylated IFNs increase host exposure to IFN and double the response seen with standard IFN preparations (521,522,523). These formulations replaced standard IFNs after their approval by FDA in 2001. Pegylation involves the attachment of a large inactive molecule (polyethylene glycol [PEG]) to a protein to reduce clearance. This process results in some variable loss of activity of the native protein that is dependent on the size and site of attachment of the PEG molecule (524). In the case of IFNs, pegylation results in a 10-fold increase in drug half-life and a corresponding decrease in clearance (525,526). It is this longer half-life that allows large doses of the drug to be administered less frequently (once weekly instead of three times per week). The PEG molecule is cleaved after binding of the complex to the IFN receptor and cleared. Short PEG molecules, such as the 12-kDa tail attached to the IFN α-2b protein, are renally cleared while longer molecules, such as 40-kDa tail on the α-2a drug, are hepatically metabolized (527). At the present time, there is no evidence that either PEG molecule has any deleterious effects.

Pegylated IFNs are more effective than standard IFN, and there is a clear dose response with increasing doses of the PEG-IFNs (521,522,523,524,525,526,527,528). Studies of monotherapy with pegylated IFN α-2a at a fixed dose of 180 μg weekly for 48 weeks resulted in an SVR rate twice that observed with the standard IFN α-2a thrice-weekly dosing control group (39% vs. 19%) (522). Similarly, studies of pegylated IFN α-2b using weight-based dosing of 1.0 or 1.5 μg/kg weekly for 48 weeks demonstrated an SVR rate twice that observed with the standard IFN α-2b control group (25% and 23%, respectively, vs. 12%) (523). It is important to point out that the study groups were not comparable and the sustained response rates cannot be directly compared; it is likely that any differences in efficacy between the two drugs, if they exist at all, are minimal.

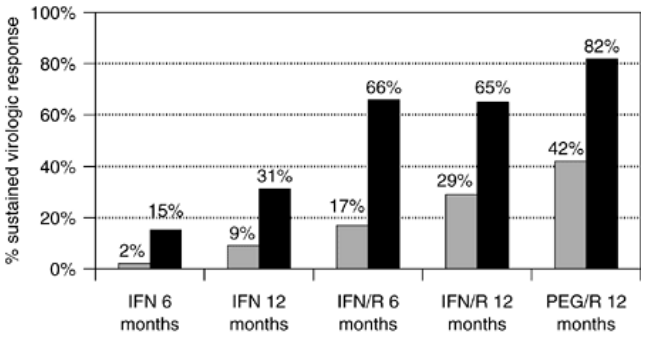
Neither pegylated IFN preparation alone has clinical efficacy approaching the combination of standard IFN and ribavirin. Therefore, the clear role of pegylated IFNs is in combination with ribavirin, and this combination is currently the standard of care for chronic hepatitis C. Large international randomized controlled studies confirmed the advantage of the pegylated combinations over standard IFN combinations (528,529). PEG-IFN α-2b 1.5 μg/kg once weekly plus 800 mg daily ribavirin led to an SVR rate of 54%, although the dose of ribavirin in this study was suboptimal (528). Sustained response was 42% in patients infected with genotype 1 and 82% in those with genotype 2 or 3 (528). In another trial, PEG-IFN α-2a 180 μg once weekly plus 1,000 to 1,200 mg of ribavirin per day resulted in an SVR rate that was 56% (529). Sustained response was 46% in patients infected with genotype 1 and 76% in those with genotype 2 or 3 (529). In both studies, tolerance including cytopenia and discontinuation of drug was similar to that of standard IFN and ribavirin, although fever, nausea, and injection site erythema were seen more commonly.

Optimal treatment regimens

The sensitivity of different HCV genotypes to IFN-based therapies varies considerably (Fig. 30.13). Therefore, determination of the virus genotype before treatment remains a critical step, and the optimal dosing regimens for the predominant genotypes have been more clearly defined (Table 30.10). For patients with genotype 1, the optimal regimen includes 1 year of PEG-IFN plus ribavirin. Although the approved dose of ribavirin is 1,000 to 1,200 mg/day for those with weight less than or greater than 75 kg, respectively (530), an extended weight-based dose ranging from 800 to 1,400 mg/day is commonly used (Table 30.11). It is not known whether such dosing improves response or is appropriate for other genotypes. Recently, some investigators have suggested that "rapid viral response" (RVR) (undetectable HCV RNA after 4 weeks of treatment) in patients with genotype 1 identifies a small subgroup (approximately 20% of treated patients) who may be treated for only 24 weeks and still achieve an SVR rate of 73% to 91% (531). Others have found a lower SVR rate with 24 weeks of treatment, so this needs to be confirmed before this regimen is made standard practice (532).

Patients with genotype 2 or 3 respond as well with doses of 800 mg/day and just 6 months of treatment as they did with higher doses and a longer duration (530). Recently, studies have suggested that some patients with genotype 2 or 3 infection who respond rapidly to

treatment can be treated for as little as 14 to 16 weeks with excellent outcomes (537).



▪ **Figure 30.13** Influence of viral genotype on sustained virologic response to interferon (IFN)-based treatment (*shaded bar*, genotype 1; *solid bar*, genotype 2 or 3). IFN/R, interferon and ribavirin; PEG/R, polyethylene glycol and ribavirin.

Patients who are infected with genotype 4 have viral response rates similar to or perhaps slightly better than those infected with genotype 1 and, like genotype 1, some achieve good responses with only 24 weeks of therapy (533). Genotypes 5 and 6 have SVR rates approaching those achieved with genotypes 2 and 3, although they require a full year of therapy (534,535).

Patient selection, drug administration, and monitoring therapy

Rational treatment management decisions are based on a clear understanding of the epidemiology and natural history of chronic hepatitis C, as well as the factors that influence the response to treatment. These decisions require that both the patient and the physician be fully informed about the disease. In the individual patient, general health factors such as age, comorbid disease, psychosocial circumstances, compliance, desire to be treated, contraindications to treatment, and financial issues must be weighed in arriving at an initial decision about whether treatment is appropriate. Counseling about transmission, natural history, treatment options, and possible treatment risks and outcomes is indicated regardless of what intervention, if any, is ultimately decided on. All should be counseled about the significant risks of alcohol use with this disease (538). Patients should also clearly understand the risks of complications should they or their sexual partners become pregnant during or shortly after the treatment course. All patients, particularly those with a history of depression, should be forewarned of the risk of depression and the need to treat it early. If treatment is not considered at this time, the importance of long-term follow-up must be emphasized. If the physician and patient agree to proceed with the treatment, assessment of the status of the disease (as determined by liver biopsy) and infection (as determined by viral genotype and HCV RNA level) should be made. This allows a more accurate estimate of the prognosis and chance of response to available treatment. It also allows the physician to use these patient characteristics that may independently influence treatment response to personalize the treatment strategy to achieve the optimal response. Although most hepatologists use only viral genotype and histology to choose the best treatment duration (539), others have recommended a more complex "a la carte" method that also incorporates gender, age, and viral level into the equation (540). Retrospective analysis suggests that such algorithms might improve

SVR rates to 50% to 83%. However, the wisdom of complicated algorithms that would, in effect, treat a higher proportion of infected cases with a longer and more costly regimen is controversial and has not been confirmed prospectively.

Table 30.10. Interferon and Ribavirin Dosing According to Viral Genotype (530,533,534,535,536)

Genotype	Interferon dose (/wk)	Ribavirin dose (mg/d)	Duration (wk)	SVR (%)
1	180 µg PEG α-2a *or* 1.5 µg/kg PEG α-2b	800–1,400 weight based	48	41–42
2	180 µg PEG α-2a *or* 1.5 µg/kg	800	24	60–84

	PEG α-2b			
3	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	800	24	60–84
4	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	1,000–1,200	48	55
5	180 μg PEG α-2a *or* 1.5 μ/kg PEG α-2b	1,000–1,200	48	64
6	180 μg PEG α-2a *or* 1.5 μg/kg PEG α-2b	1,000–1,200	48	63

SVR, sustained virologic response; PEG, polyethylene glycol.

Table 30.11. Dosing Guidelines for Combination Therapy with Pegylated Interferon and Ribavirin (536)

Body weight in kilograms (pounds)	Peg-IFN α-2a dose (μg)	Peg-IFN α-2b dose (μg)	Ribavirin dose (≥13 mg/kg)
<40 (<88)	180	50	800
40–50 (88–100)	180	64	800
50–65 (112–141)	180	80	800
65–75 (143–165)	180	112	1,000
76–85 (167–187)	180	120	1,000
86–104 (189–229)	180	150	1,200
>105 (>231)	180	150	1,400

- U.S. Food and Drug Administration–approved dosing for pegylated IFN α-2b and ribavirin is 1.5 μg/kg once per week and 800 mg/d in divided dose, respectively (454,460).
- Traditional doses of ribavirin based on licensed recommendations with nonpegylated regimens are 1,000 mg/day if body weight is less than 75 kg or 1,200 mg if weight is more than 75 mg.

Baseline assessment of liver tests, complete blood counts, and HCV RNA level are important to later determine treatment response and drug-related toxicity. The patient should be instructed in injection techniques and forewarned about what drug-related symptoms are expected. Treatment tolerance is improved if the patients are educated about the potential side effects of therapy and what they might expect. Some easy measures such as evening dosing, exercise, adequate hydration, and use of acetaminophen at the time of each IFN dose will reduce anxiety, side effects, and noncompliance. Reinstitution of antidepressants should be considered in patients with a significant past history of depression. Physician extenders such as nurses, pharmacists, and commercial treatment support services are extremely helpful in this respect.

Standard treatment of chronic hepatitis C should consist of the combination of pegylated IFN α- 2b (PEG-

Intron™, Schering Plough, Kenilworth, NJ, at a dose of 1.5 μg/kg body weight) or pegylated IFN α-2a (Pegasys, Roche, Nutley, NJ, at a fixed dose of 180 μg) administered subcutaneously once per week and 13 to 15 mg/kg (minimum dose, 800 mg) of oral ribavirin per day in divided doses (Table 30.11). Pegylated IFN α-2b is dosed on the basis of body weight because it has a larger volume of distribution than pegylated IFN α-2a (20 L vs. 8 L), which has a volume of distribution approximately equivalent to plasma volume and can therefore be administered as a fixed dose (529). The listed doses of ribavirin are based on retrospective analyses of ribavirin dose response in clinical trials (528,529,530,536,540). Although this extended weight-based dosing method has become common, the advantage of extended dosing method appears to be marginal in comparison to the previous limited weight-based dose (1,000 or 1,200 mg) (536). Nonetheless, a recent study demonstrating that even higher doses of ribavirin may improve response rates justify the use of this dosing method (541,542). Viral genotype determines the duration of therapy (Table 30.10).

Monitoring response and potential drug toxicity is essential. Symptoms related to treatment rarely necessitate dose adjustments. However, hematologic alterations, particularly anemia, can be significant and clinically important during the first few weeks. Therefore, blood counts including hemoglobin, white count, differential, and platelet count should be repeated 2 and 4 weeks after starting therapy. Transient IFN dose reduction is indicated only for a white count less than 1,500/mL, a neutrophil count less than 750/mL, or platelets less than 50,000/mL. Ribavirin should be reduced if the hemoglobin level falls to less than 10 g/dL. The amount of dose reduction required to reverse cytopenia has not been established. Although the labeling of the drugs calls for reducing doses by half, this is usually not necessary. Temporary dose modifications are common in patients treated with combination therapy. In fact, in large controlled trials dose modifications were required at least transiently in 34% to 42% of patients, respectively (510,511,528,529). Between 8% and 13% of patients require reduction of the ribavirin dose for anemia, usually during the first 4 weeks of treatment (543). Ribavirin-induced anemia is dose dependent, and therefore, anemia usually stabilizes or improves with dose reduction. Occasionally, transfusion or support with erythropoietin is necessary, although it generally takes 4 to 8 weeks before the hemoglobin level increases after erythropoietin is started (544). The hemolytic anemia is accompanied by a vigorous reticulocytosis that usually serves to maintain stable levels of hemoglobin after the first few weeks of treatment and result in its return to baseline within 4 weeks of stopping treatment. Approximately 15% to

20% of patients treated with pegylated IFN require dose reductions for neutropenia (528). Growth factor support is rarely required. Significant thrombocytopenia necessitating dose reduction is uncommon because the anemia caused by ribavirin induces a reactive thrombocytosis. Therefore, platelet counts tend to remain relatively stable throughout combination therapy, even when the pretreatment count is low. IFN should be permanently stopped only if symptoms are incapacitating, the absolute neutrophil count is less than 500/mL, or the platelet count is less than 25,000/mL. Discontinuation of therapy for cytopenia is uncommon if patients have been monitored and dose adjusted appropriately. It is extremely important that treatment not be stopped prematurely or for decreases in blood counts that do not meet the criteria stated earlier. Inappropriate dose reduction and discontinuation significantly reduces the likelihood of a treatment response. Early discontinuation of treatment can reduce the likelihood of a sustained treatment response by 80% (545). Management of other side effects is discussed later.

Side effects of treatment

The safety and tolerability of combination therapy have been reviewed in detail elsewhere and will only be highlighted here (543,546). Overall, IFN-based therapies are reasonably well tolerated. Most patients experience flu-like side effects including fatigue, fever, headache, myalgia, and arthralgia (528,543). These are most severe when treatment is initiated and often abate to a large degree as treatment is continued. Gastrointestinal symptoms including nausea, vomiting, or diarrhea occur in about a third of patients but are rarely severe. Psychiatric symptoms such as depression, impaired concentration, irritability, and insomnia occur in about a third of cases but are also common in untreated patients with chronic hepatitis C (510,528,547). Dermatologic signs and symptoms occur in about a quarter of patients, but injection site erythema is seen in 30% to 60% of cases and is more common and pronounced with pegylated IFNs (528).

As described in the preceding text, ribavirin causes a predictable dose-related hemolysis. Therefore, the drug should be used with great caution or avoided completely if there is preexisting anemia, a hemolytic disorder, coronary artery disease, or hypoxia. Because ribavirin is renally excreted, it can cause profound hemolysis in patients with renal failure and should generally be avoided. Careful consideration should be given to the potential effects of an acute anemia in each patient in whom combination treatment is considered. The mean fall in hemoglobin is level 2 to 3 g/dL (510,511,517). The decline occurs gradually during the first 4 weeks of treatment and the hemoglobin level usually remains relatively stable thereafter.

Severe adverse events, including severe psychiatric symptoms, suicide attempts, and profound cytopenia, are extremely uncommon, being reported in fewer than 1 in 1,000 treated cases (546). Development of immune-mediated disorders such as thyroid disease, diabetes, dermatologic conditions, neuropathy, and

other autoimmune-like signs was seen in about 1% in a large retrospective series (546). Development of autoantibodies is not necessarily associated with autoimmune disease. Autoantibodies are common in patients with HCV infection and may be more common during IFN treatment (133,548). Finally, ribavirin has embryotoxic and teratogenic effects in animals and should be avoided in patients with childbearing potential unless adequate contraception is assured. Trial and postmarketing surveillance data suggests that most patients or spouses of patients who become pregnant during or within 6 months of treatment will spontaneously abort if the pregnancy is not otherwise terminated (GL Davis, unpublished data, 2002).

Assessing treatment response

Treatment responses are defined by changes in the HCV RNA level during and after treatment; these are listed and described in Table 30.12. Early studies also utilized serum ALT level to assess the response to therapy, but virologic endpoints are now most appropriate. Serum ALT level does not always reflect on treatment response because ribavirin may normalize serum ALT level in the absence of a virologic response and ALT level may occasionally be elevated despite a virologic response, especially in those receiving pegylated products (517,528,529).

The implications of RVR are described in the preceding text, but this is a new concept and requires confirmation before influencing treatment duration in most patients. Early virologic response (EVR) is used to assess nonresponsiveness to treatment during the first weeks of therapy. EVR is based on the concept that the slope of the second-phase decline in HCV RNA levels during treatment correlates with the likelihood of eventual virus clearance (82,549). Obviously, it requires a quantitative HCV RNA assay with a wide dynamic range to assess this decline (See "Diagnostic Tests"). Genotype 1–infected patients who do not achieve at least a 2-log reduction (99%) in HCV RNA level after 12 weeks of treatment have less than 1% chance of reaching an SVR with continued therapy (Fig. 30.14) (550). This has served to justify discontinuation of therapy after 12 weeks in the 20% or so of patients without EVR. Patients with genotype 2 or 3 almost always reach an EVR, so it is usually not helpful to assess HCV RNA levels in them during treatment (550).

Measurement of HCV RNA at the end of therapy is helpful in identifying those in whom the virus has

cleared (end-of-treatment response) and who require subsequent screening to confirm the durability of the response. Approximately 15% of patients with end-of-treatment response will relapse during the first few months after treatment is stopped (528,529). SVR is confirmed by the absence of detectable HCV RNA by a sensitive molecular test 6 months after completing therapy. SVR is the major goal of treatment and is durable.

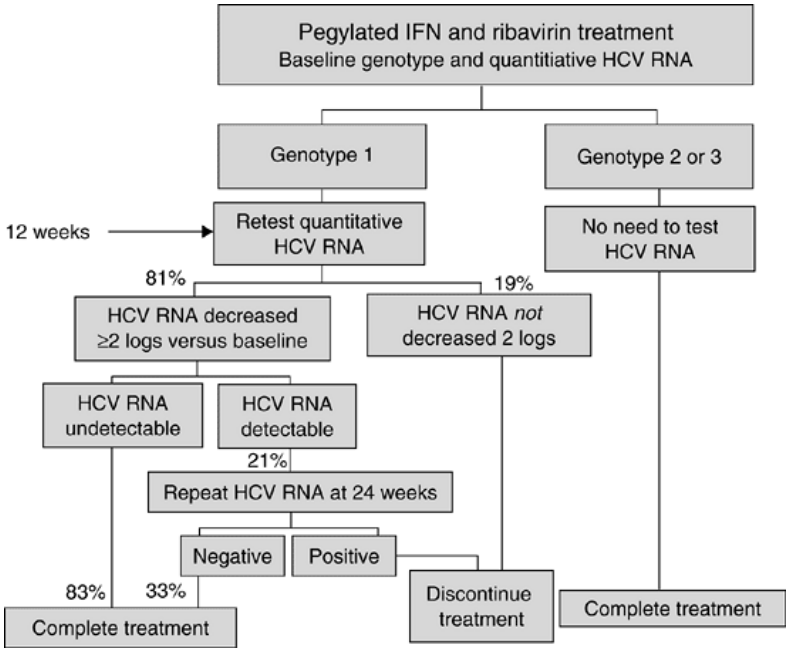
Table 30.12. Definitions of Treatment Responses

Response	Time to assess	Definition	Implication
RVR	4 wk	HCV RNA undetectable by PCR or TMA	Higher chance of SVR; may respond as well with only 24 wk of treatment
EVR	12 wk	HCV RNA decreased by ≥two logs from baseline or HCV RNA undetectable	Failure to achieve EVR associated with almost no chance of SVR and treatment can usually be stopped
ETR	End of treatment	HCV RNA undetectable by PCR or TMA	On treatment response; observe for SVR
SVR	24 wk after treatment	HCV RNA undetectable by PCR or TMA	Eradication of virus

RVR, rapid viral response; HCV, hepatitis C virus, RNA, ribonucleic acid; PCR, polymerase chain reaction; TMA, transcription-mediated amplification; SVR, sustained virologic response; EVR, early virologic response; ETR, end-of-treatment response.

Clinical implications of sustained virologic response

SVR is the primary goal of treatment. It is durable and connotes eradication of infection (551). Furthermore, SVR results in long-term histologic improvement with fibrosis regression at a rate of 0.28 Metavir units per year (552,553,554,555). By contrast, nonresponders to IFN-based therapy continue to have progression of fibrosis at a rate of 0.10 Metavir units per year (555). It is reasonable to assume that these benefits would translate into a reduction in disease-related morbidity and mortality. Indeed, patients with fibrosis or cirrhosis who achieve SVR have gradual improvement in quantitative hepatic function, reduction in portosystemic shunting, significant reduction in the risk of developing HCC, and improved survival (556,557).



▪ **Figure 30.14** Algorithm for determining early virologic response. IFN, interferon; HCV, hepatitis C virus; RNA, ribonucleic acid.

Treatment in special patient groups

Fibrosis or cirrhosis

The presence of bridging fibrosis or cirrhosis negatively impacts treatment response to IFN-based regimens. SVR among patients with stage 3 or 4 fibrosis is 41% to 44% compared to 54% to 55% in those with stage 0 to 2 (528,529).

The justification for treating patients with advanced fibrosis is clear because these patients have a greater risk of progressing to develop HCC or complications of cirrhosis. Patients with fibrosis or cirrhosis who achieve SVR have a reduced risk of developing these complications (557).

Higher doses of IFN may overcome some of the lack of response to IFN. In a trial using a dose of 3.0 μg/kg of pegylated IFN α-2b, SVR was the same in patients with and without advanced fibrosis, although this was not the case with the standard IFN dose (558). Finally, it has been suggested that chronic low-dose IFN might also delay progression of fibrosis and reduce complications of liver disease in patients with fibrosis or cirrhosis who have failed to reach an SVR with standard antiviral therapy. This is currently being evaluated in prospective clinical trials (See "Viral Nonresponders").

Treatment of patients with decompensated cirrhosis can be considered in the hope of eradicating infection before liver transplantation. SVR in this group usually prevents recurrence of HCV infection after transplantation (559). However, such treatment is extremely difficult. Most patients have cytopenia that prevents them from receiving the optimum doses of medications, and severe complications usually require dose reductions or discontinuation of therapy (559,560). These problems may be partially avoided by initiating treatment at low doses and escalating as tolerated (559). Despite the problems, SVR is possible in approximately 25% of patients. Treatment in this group of patients is best managed by experienced transplantation hepatologists.

High viral load

High levels of HCV RNA are associated with a decreased chance of clearing virus, at least with genotype 1 infection (528,529). SVR in patients with more than 2 million copies per milliliter achieved SVR in 30% to 39% compared to 56% to 68% in those with lower levels (528,529). Furthermore, patients with low levels of HCV RNA before treatment are also more likely to reach an RVR that may allow them to discontinue treatment early (531). It does not appear that higher doses of IFN are able to overcome the impaired response in patients with high viral loads.

African Americans

SVR rates have consistently been lower in African Americans than in whites in trials of IFN-based therapy. Recent studies with pegylated IFN and ribavirin in previously untreated patients with genotype 1 infection observed SVR rates of 19% to 26% in African Americans versus 39% to 52% in whites (561,562). Several factors including the higher proportion with genotype 1, higher body weight, and a slower decline in HCV RNA in viral kinetic studies might at least partially explain this observation. However, the apparent ability of higher doses of IFN or ribavirin to improve responses suggests that the explanation may lie in the impaired ability of intracellular antiviral mechanisms to be turned on at standard doses of these medications (558,563).

Human immunodeficiency virus–hepatitis C virus coinfection

HCV infection is common in HIV-infected individuals (564). Coinfected patients tend to have high HCV RNA levels, and some studies suggest that they have more severe liver disease, more rapid disease progression, and a higher prevalence of hepatic fibrosis (455,565,566,567,568). Liver disease due to hepatitis B, hepatitis C, or alcohol is second only to acquired immunodeficiency virus as a cause of death in patients with HIV (569). Several clinical trials of pegylated IFN and ribavirin have recently been reported (570,571,572). Treatment responses vary considerably in these studies because of differences in design and compliance. However, SVR in genotype 1 patients was low (14% to 29%) while it was reasonably intact in genotype 2 and 3 subjects (62% to 73%). Overall, SVR rates appeared to be 10% to 15% below what would be expected in an HIV-negative population. Several points are worth making. First, SVR is possible in the HIV–HCV coinfected patient and treatment should be considered. Second, failure to achieve EVR reliably predicts lack of response in this group and this should be discussed before treatment is started because the treatment goals are different from those of HIV. Finally, patients receiving highly active antiretroviral therapy (HAART) or with low CD4 counts appear to be more likely to experience adverse effects from treatment, including drug hepatotoxicity (564,573). Severe hepatotoxicity, especially related to ritonavir, is almost four times higher in patients with HIV who are coinfected with HCV, having a risk of approximately 12% (573). However, 88% do not develop severe hepatotoxicity. Furthermore, ribavirin may increase phosphorylation of zidovudine and dideoxyinosine and potentiate toxicity of these drugs (574,575). However, despite a case report implicating ribavirin in multiorgan failure and lactic acidosis, most HIV physicians have not found the potential drug interaction to be a clinical problem.

Obesity and fatty liver disease

Obesity and hepatic steatosis are both associated with a reduced response of HCV-infected patients to IFN-based treatments (528,529,576). For genotype 1 and 2, hepatic steatosis is associated with a fall in SVR from 57% to 25% and 96% to 86%, respectively (576). Genotype 3 infection induces steatosis in and of itself, and

this is not associated with reduced SVR. The mechanism(s) by which obesity or steatosis reduce(s) SVR is (are) not known, but it has recently been suggested that it may be related to insulin resistance and hyperinsulinemia. Sanyal et al. studied the effect of insulin on the action of IFN in an HCV replicon system composed of a Huh-7 hepatocellular carcinoma cell line stably transfected with full-length HCV RNA (577). IFN predictably inhibited HCV replication in this model. However, addition of insulin to the media resulted in a significant increase in HCV replication and a marked blockade of IFN's ability to decrease HCV RNA. The mechanism of insulin interference appeared to be impairment of activation of the STAT pathway with resulting blockade of the IFN-mediated increase in protein kinase R and IRF-1. Other mechanisms may also be involved.

Acute hepatitis C

Acute hepatitis C has a high propensity for progression to chronicity in the absence of antiviral therapy. Over the last 15 years, there have been numerous studies assessing the efficacy of IFN for the prevention of chronic infection. These studies vary considerably in design, type and dose of IFN, use of ribavirin, duration of therapy, and presence of controls. Nonetheless, it is clear that acute hepatitis C is extremely sensitive to the effects of IFN and most treated patients resolve the infection regardless of the regimen utilized (397,398,399,509,578,579,580,581,582). More recent studies have utilized either daily doses of a standard IFN or weekly doses of pegylated IFN for 4 to 24 weeks (579,580,581,582). SVR rates ranged from 75% to 98%. One study randomized patients to immediate treatment or a delay until 1

year after onset (582). Chronicity was reduced from 60% to 13% by early intervention.

Although these results are quite encouraging, treatment of patients with acute HCV infection must be considered in the proper perspective. The incidence of acute hepatitis C in the United States has fallen dramatically to about 28,000 infections per year (243). Only a small proportion of these cases are recognized by either the patient or physician. Furthermore, prospective surveillance to identify acute infection in those at risk (e.g., intravenous drug users) is impractical. Therefore, most cases of acute hepatitis will never come to medical attention. Nonetheless, treatment of identified cases with 6 months of pegylated IFN should be strongly considered. There is no data suggesting that ribavirin is required.

Extrahepatic manifestations

Mixed cryoglobulinemia is the most common extrahepatic manifestation in patients with chronic hepatitis C. It typically manifests as cutaneous vasculitis, glomerulonephritis (GN), neuropathy, or systemic vasculitis (583,584). IFN alone, or in combination with ribavirin, is able to suppress cryocrits in most patients with mixed essential cryoglobulinemia, and treatment may be indicated solely on the basis of the presence of clinical complications of cryoglobulinemia, regardless of the presence or severity of the liver disease (583,584,585,586,587). The fall in cryocrit observed during treatment usually correlates with a drop in serum HCV RNA levels, normalization of complement levels, and clinical improvement (583,586,587). However, polyneuropathy appears to be relatively resistant to treatment (585,586). Although results in this small series vary considerably, SVR after discontinuation of IFN-based treatment appears to be lower than that in patients without cryoglobulinemia (587).

GN has been associated with chronic HCV infection (588,589). Most of these cases present with proteinuria, which may reach nephrotic syndrome range, and have cryoglobulinemia (588,589,590). The histologic lesion is usually one of membranoproliferative GN, so-called cryoglobulinemic GN, although other histologic forms such as mesangial proliferative GN (usually IgA nephropathy) and membranous GN may also occur (588,589,590,591,592). In contrast to membranoproliferative GN, the latter two histologic lesions are typically not associated with cryoglobulinemia (588,591). Membranous GN responds poorly, if at all, to IFN (588,592). Furthermore, a recent report cautions that GN not caused by HCV may worsen under IFN treatment (593). Finally, ribavirin is renally excreted and should be used with great caution, if at all, in patients who have developed significant renal insufficiency as a consequence of their GN (578).

Post-transplantation

Complications of chronic hepatitis C including HCC and decompensated cirrhosis are the most common indication for liver transplantation, accounting for more than 40% of transplantations performed in the United States and Europe. If HCV RNA is detectable at the time of transplantation, infection will always recur (458). HCV RNA levels increase rapidly after transplantation as a consequence of immunosuppression (594,595,596,597). These patients often have very high HCV RNA levels, which may result in sometimes rapidly progressive chronic hepatitis, or, less commonly, an aggressive cholestatic hepatitis, which leads to liver failure (458,598,599,600,601).

Several options for treatment should be considered in the setting of liver transplantation including treatment of listed patients awaiting transplantation, preemptive therapy for patients shortly after transplantation before histologic recurrence is noted,

or treatment once recurrent disease is apparent (458,559,560,602,603). Treatment of such patients is predictably difficult regardless of which strategy is chosen because most patients have genotype 1 infection and high viral loads, have previously failed to respond to or tolerate IFN-based therapy, often have preexisting cytopenia and renal insufficiency, and have drug interactions that increase the risk of cytopenia or symptoms. Although antiviral treatment is successful in some patients with recurrent hepatitis C, it is extremely difficult to administer and requires dose reductions in most cases (458,604). Furthermore, acute and chronic rejection may ensue during or after treatment (604). Nonetheless, approximately 30% of treated patients can achieve SVR if both the patient and physician are committed to therapy (458,603).

IFN-based antiviral therapy should generally be avoided in recipients of other solid organ transplants because of the risk of rejection (605). However, treatment appears to be safe in autologous and allogeneic bone marrow transplant recipients (606).

Normal alanine transaminase level

Approximately 15% to 20% of patients with chronic HCV infection have persistently normal serum ALT levels (264). Although most of these patients have some degree of inflammation on liver biopsy, most studies have shown that few have significant fibrosis. In one review of 11 published studies, cirrhosis was found in only 0.3% (607). Others have reported fibrosis in up to 10% (608). However, in the absence of alcohol use, the rate of fibrosis progression is very low in patients with persistently normal serum ALT levels (609,610). Virologic response to IFN-based therapy appears to be the same as that in

patients with elevated ALT levels (611). Therefore, treatment can be considered on an individual basis in these patients, balancing the cost and difficulty of therapy with the estimated likelihood of progression. Although it may be hard to justify the cost and side effects of therapy in many patients, it is easy to make an argument for treating patients with hepatic fibrosis or genotypes 2 and 3.

Viral relapsers

Virologic relapse occurs in 45% to 80%, 35% to 40%, and 15% of those who lose detectable HCV RNA during IFN monotherapy, standard combination therapy, or pegylated combination therapy, respectively (186,510,511,528,529). There is little or no benefit in retreating patients with the same treatment regimen (612,613). However, treatment of relapsers to monotherapy with higher doses or a longer duration of IFN, or combination therapy, may achieve a sustained response (614,615,616,617). However, patients previously treated with suboptimal regimens should always be given the current standard of care regimen if they are retreated. A recent study found that half of patients who relapsed after standard IFN and ribavirin achieved SVR when retreated with pegylated IFN and ribavirin (618).

Viral nonresponders

Mathematically, one would predict that approximately 10% to 20% of viral nonresponders to IFN monotherapy or standard combination therapy might achieve a sustained response when retreated with pegylated combination therapy. Indeed, prospective trials have confirmed this to be the case (618). However, much of the published literature is quite confusing because of differences in definitions of nonresponse (some include relapsers and partial responders), different retreatment regimens, and inclusion of high proportions of patients with factors that favor response (i.e., patients without cirrhosis, those with genotypes 2 or 3, and those with low HCV RNA levels) (619). Furthermore, the reasons for nonresponse are often not given in such studies. Many patients fail to respond to treatment because of incorrect dosing, early and sometimes inappropriate dose reductions or discontinuations, and poor side effect management. These patients might be expected to respond better to retreatment than those who were given full dosages whose virus was refractory to the drugs. Indeed, patients who have partial HCV RNA response to the initial course of treatment are far more likely to respond to retreatment than those whose virus level did not change (620,621).

Several strategies for improving response to retreatment of nonresponders have been proposed. Several trials with high-dose IFN induction therapy were unsuccessful. A longer duration of treatment might be helpful in patients with slow and partial response. Berg et al. found that although the overall response was similar in patients treated for 48 or 72 weeks, those who had EVR but still had low levels of virus (<50,000 IU/mL) were most likely to benefit from prolonged treatment (622). Another study randomized genotype 1 patients who remained PCR positive at week 4 to 48 or 72 weeks (623). This study found a higher SVR rate among those who received the longer course of treatment, but the study was hard to interpret because it utilized a suboptimal dose of ribavirin and the overall SVR rate was accordingly low. More recently, a few studies have suggested that high doses of antiviral drugs throughout the treatment course might be successful in achieving SVR in patients who were resistant to the effects of standard doses. Leevy et al. initiated 48 weeks of high-dose daily consensus IFN and ribavirin in patients who failed to achieve an early viral response (563). One would expect that almost no patient without EVR would achieve SVR with continued treatment with the initial doses (550). Nonetheless, the SVR rate with

this intensive regimen was similar to that with treatment of naïve subjects (27% in African Americans and 41% in whites). These data are intriguing and suggest that higher drug doses throughout treatment might be effective in some difficult-to-treat subgroups. Indeed, Gross et al. reported the results of the REtreatment of NonrEsponders with Weight-based dosing (RENEW) study in which 704 subjects who had failed to clear HCV RNA during a previous treatment course were treated with either 1.5 μg/kg or 3.0 μg/kg of pegylated IFN α-2b in combination with weight-based ribavirin (800 to 1,400 mg/day) for 48 weeks (558). SVR was significantly higher in the high-dose group (17% vs. 12%). Importantly, while SVR was lower in African Americans and patients with advanced fibrosis who received a standard dose of pegylated IFN, these differences were not seen in the high-dose group. Similarly, Shiffman et al. reported that high-dose ribavirin significantly reduced relapse and as a result almost doubled the SVR rate, particularly in difficult-to-treat patients such as African Americans (624).

Another strategy for retreating nonresponders is long-term IFN maintenance. It is clear that some patients who do not clear HCV RNA during treatment have a reduction in hepatic inflammation and fibrosis (186,426,510,511,528,529). However, it is not known whether this effect persists with maintenance therapy or whether it might result in a lower risk of disease complications such as liver decompensation or HCC. Shiffman et al. found that fibrosis could be reduced with long-term IFN administration in the subset of patients who demonstrated early histologic improvement despite viral persistence (625). Everson reviewed the results of three trials that administered IFN treatment of patients with cirrhosis (626). The overall incidence of HCC was 15% in untreated patients, 4% in IFN nonresponders, and 0% in sustained responders (626,627,628,629,630). Although the accumulated data suggests that IFN might provide a beneficial long-term effect, no prospective studies have confirmed any benefit to date. However, three trials are currently under way to examine the efficacy of long-term

pegylated IFN in preventing progression of fibrosis and development of decompensation (the federally sponsored Hepatitis C Long-term Treatment against Cirrhosis (HALT-C) study and the pharmaceutical company–sponsored Colchicine versus PEG-INTRON Long-Term [COPILOT] and Evaluation of PEG-INTRON in Control of Hepatitis C Cirrhosis [EPIC3] studies).

In summary, retreatment should be strongly considered in patients who previously received IFN monotherapy or standard IFN in combination with ribavirin. This is particularly true in patients who already have advanced fibrosis. Retreatment of patients who failed to respond to pegylated IFN and ribavirin is unlikely to be beneficial and cannot be recommended. Several studies with high-dose antiviral therapy need to be confirmed before these aggressive regimens are routinely used. To date, there is no good evidence to justify maintenance IFN therapy outside the clinical trials.

Nonprescription Agents Used for Chronic Hepatitis

The use of alternative medicines is increasingly common in the United States and Europe, with nearly half of surveyed adults using some form of these agents (631). Several agents, including α-tocopherol, bayberry, blessed thistle, milk thistle, blue flag, dandelion root, fringe tree bark, gentian, yellow dock, and various Chinese herbal remedies, have been touted as effective remedies for chronic hepatitis in the lay literature, although there are little data available to support their use. Silymarin, commonly referred to as *milk thistle*, is the most popular of the herbals and is used by approximately 12% of patients with chronic hepatitis C, although this is probably a significant underestimation because nearly 40% of patients fail to report the use of such compounds to their physicians (632). The active ingredients in milk thistle are the flavanoid silymarin and its main structural component silybin. Animal and cell culture studies have demonstrated that these compounds inhibit the lipoxygenase pathway and have antioxidant properties that diminish toxicity induced by a variety of hepatotoxins including *Amanita phalloides*, acetaminophen, and allyl alcohol if the drug is administered before toxin exposure (633,634). However, both silymarin and silybin have been shown to induce cell damage in cell culture systems (635). The popularity of milk thistle among patients with liver disease must be attributed to word-of-mouth, lay literature, and highly effective although misleading Internet marketing ("nature's premier herbal liver tonic with specific protective benefits for liver tissues"; "supports the production of new liver cell to replace the old or damaged ones") because the compound has repeatedly been shown to be ineffective for the treatment of chronic hepatitis C (636,637,638,639). Milk thistle does appear to be well tolerated for short periods except for occasional gastrointestinal symptoms and rash (640,641). Nevertheless, all herbal and nonprescription agents should be used with extreme caution, especially in patients with significant liver disease, because the safety profiles of these remedies have not been critically studied.

Future Treatment Options

Future options for combination therapy may include antibodies or envelope inhibitors to prevent binding to receptors and cell entry, oligonucleotides such as interfering RNA or antisense molecules to prevent

attachment to the endoplasmic reticulum and translation, protease inhibitors to prevent processing of the translated polypeptide, polymerase inhibitors to prevent production of viral RNA progeny, glucosidase inhibitors to prevent assembly and secretion of virions, immune-modulating agents to enhance host response to infection, and antifibrotics that decrease the fibrogenic response to inflammation (Fig. 30.2). The most promising agents in development are the inhibitors of intracellular HCV replication. Development of these drugs has been facilitated by descriptions of the three-dimensional structures of the NS3 protease, helicase, and RNA-dependent RNA polymerase (642,643,644). These enzymes are unique to HCV and significantly different from the HIV protease. Several protease inhibitors have entered trials in humans and show significant promise (645,646). These agents block the ability of HCV to cleave the nonstructural proteins from the large translated polypeptide. They are orally bioavailable, well tolerated, and potent inhibitors of HCV replication. Furthermore, as predicted, these drugs appear to increase the sensitivity of infected cells to IFN, perhaps through reduction of NS3 activity (107,114,647). All agents to date have short half-lives and must be dosed frequently to maintain inhibitory trough levels, and this may represent a temporary obstacle to their clinical use. The HCV polymerase replicates the negative-strand template and produces positive-strand progeny RNA for incorporation into new virions. Several HCV polymerase inhibitors are in development, and one agent is well along in development in humans (648). Although the drug has only modest antiviral activity by itself, it too acts in synergy with IFN and thus far in early clinical trials has not selected drug-resistant variants.

The major challenge as these new agents are developed and tested will be figuring out just how to integrate them into current treatment regimens to increase efficacy, improve tolerability, or both. Given the ability of our current IFN-based therapy to eradicate infection in more than half of treated patients, it is likely that IFNs will remain the foundation of future therapeutic regimens for some time.

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Chapter 31

Autoimmune Hepatitis

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Key Concepts

- Autoimmune hepatitis (AIH) represents a chronic inflammatory disorder of the liver, involving loss of tolerance to hepatic tissue antigens.
- AIH is characterized by a predominantly periportal, lymphoplasmacytic infiltrate associated with hypergammaglobulinemia and circulating autoantibodies.
- Epidemiologically and clinically distinct subtypes are defined by different autoantibody patterns.
- AIH prevalence is highest among females, displaying an immunogenetic association with human leukocyte antigens (HLA)-A1-B8-DR3 or HLA-DR4 haplotypes.
- Extrahepatic immune-mediated syndromes are frequently found.
- The pathogenic mechanisms initiating chronic inflammation in AIH are incompletely understood. Genetic factors, molecular mimicry, dysregulated apoptosis, and immune response mechanisms have been implicated to play a role.
- AIH diagnosis is based on both the presence of characteristic laboratory features and histology, as well as the exclusion of other hepatobiliary diseases, facilitated by the revised AIH diagnostic score.
- In most cases AIH responds well to immunosuppressive therapy.
- Standard treatment consists of corticosteroids alone or in combination with azathioprine.
- If standard treatment fails, alternative drugs such as mycophenolate, cyclosporin A, tacrolimus, or cyclophosphamide may be considered.
- In refractory cases with chronic liver failure, liver transplantation remains the ultimate therapeutic option. However, AIH recurrence may complicate the course after liver transplantation.

History and Definition

First reports of severe icteric liver disease associated with hyperproteinemia were published in the 1930s and 1940s. In 1950 Waldenström described chronic hepatitis with hypergammaglobulinemia in young women, leading to jaundice and liver cirrhosis (1). This new entity of liver disease was confirmed by several authors

worldwide. Because of serologic similarities with systemic lupus erythematodes the term *lupoid hepatitis* was introduced in the mid-1950s.

Nowadays, autoimmune hepatitis (AIH) represents a chronic, mainly periportal hepatitis with lymphocytic infiltrates, plasma cells, and piecemeal necroses. It is characterized by a female predominance, hypergammaglobulinemia, circulating autoantibodies, and a good response to immunosuppressive treatment (2). Serologic detection of autoantibodies is one of the distinguishing features leading to the classification of AIH

into subgroups (Table 31.1). AIH type 1 represents the most common form of AIH and is characterized by the presence of antinuclear antibodies (ANAs) and/or anti–smooth muscle antibodies (SMAs). The target nuclear autoantigens of type 1 AIH are unknown and presumably heterogeneous. Characteristic antibodies of AIH type 2 are liver–kidney microsomal (LKM-1) antibodies directed against cytochrome P-450 CYP 2D6 and with lower frequency against uridine-5′-diphosphate-glucuronosyltransferase (UGT) (3,4). AIH type 3 is characterized by autoantibodies against soluble liver antigen and liver–pancreas antigens (SLA/LP), which are directed toward the UGA-suppressor transfer ribonucleic acid (tRNA)-associated protein (5,6). The clinical and epidemiologic features of AIH type 1 and 3 are similar. Therefore, some authors pool both subgroups under type 1 subgroup.

Table 31.1. Clinical Characteristics and Distinguishing Features of the Subclasses of Autoimmune Hepatitis

Clinical features	AIH type 1	AIH type 2	AIH type 3
Diagnostic autoantibodies	ANA/SMA	LKM-1	SLA/LP
Target antigen	Unknown	P-450 CYP 2D6 UGT	UGA-suppressor tRNA-associated protein
Percentage of prevalence of all AIH types (%)	80	20 in Europe 4 in the United States	<20
Main age of manifestation	Bimodal (16–30 y and >50 y)	Pediatric (2–14 y)	20–40 y
Extrahepatic associated diseases (%)	41	34	58
HLA association	B8, DR3, DR4	B14, DR3, C4AQO	Unknown

Progression to cirrhosis (%)	45	82	75

AIH, autoimmune hepatitis; ANA, antinuclear antibody, SMA, smooth muscle antibody, LKM, liver–kidney microsome; SLA/LP, soluble liver antigen and liver–pancreas antigens; UGT, uridine-5′-diphosphate-glucuronosyltransferase; tRNA, transfer ribonucleic acid; HLA, human leukocyte antigen.

Epidemiology

Originally described in white northern Europeans and North Americans, AIH has a worldwide distribution. The disease affects 100,000 to 200,000 persons in the United States (7) and accounts for 2.6% of transplant recipients in Europe (8) and 5.9% in the United States (9). In northern Europe the prevalence is estimated at 170 cases per million.

AIH type 1 (classical AIH) represents the most common form of AIH, whereas AIH type 2 and 3 are rare entities (10,11). AIH type 2 displays a regionally variable prevalence, with very low numbers (approximately 4%) in the United States but accounting for up to 20% of AIH cases in western Europe (10,12). In cases of ANA, SMA, and LKM negativity, which are present in 10% of AIH cases, measurement of SLA/LP autoantibodies is justified to recognize patients with AIH and to decrease the possibility of misdiagnosis (13).

Pathophysiology

Autoimmunity is characterized by T cell–dependent immunopathologic responses to auto-/neoantigens, leading to inflammatory tissue injury. An autoimmune response can be triggered by the human leukocyte antigen (HLA) class II–dependent presentation of specific antigenic peptides to T cells through antigen-presenting cells (APCs). As a response to cytokines, the exposed T cells become activated and differentiate into T_H1 or T_H2 cells. Proinflammatory cytokines are believed to play an important role in the initiation of the autoimmune response (14). Because of cytokine activation, autoimmune diseases are often observed in associated viral or bacterial infections.

Another mechanism is called *molecular mimicry*. For instance, the B-cell epitope of cytochrome P-450, which is targeted by LKM-1 autoantibodies in AIH type 2, shares sequence homology with the herpes simplex virus antigen IE 175 (3).

Under normal circumstances, the immune response is tightly regulated. T- and B-cell homeostasis, as well as the removal of autoreactive T cells, are regulated by apoptosis. The failure of control by apoptosis may therefore contribute to the initiation and perpetuation of AIH and autoimmune overlap syndromes (15). Deranged apoptosis may influence both the inability to kill autoreactive cells or the induction of autoimmunity against cellular constituents.

Genetic factors are implicated to play a role in the susceptibility to AIH. In particular, polymorphisms of the genes influencing lymphocyte homeostasis, such

as the tumor necrosis factor-α (TNF-α) promoter gene, the complement factor C4 gene, and the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene, contribute to increased susceptibility to AIH (16). One hypothesis suggests that inheritance of specific HLA class II alleles modified at critical sites provides one of the crucial

steps for the development of AIH. The relevance of genetic alterations for AIH is further underlined by the observation that chronic hepatitis occurs in 10% to 18% of patients with autoimmune polyendocrine syndrome type 1 (APS1), an autosomal recessive disorder that is caused by mutations in a single gene (AIRE) and is characterized by various autoimmune diseases mainly affecting the endocrine glands (17).

Clinical Presentation of Autoimmune Hepatitis

Patients with AIH type 1 clinically resemble those with AIH type 3 with respect to age and sex distribution, HLA antigen profile, inflammatory activity, and response to therapy (18). In contrast, the differences between AIH type 1/3 and AIH type 2 are more apparent. In AIH type 2, patients are younger and more frequently display an acute onset of hepatitis with a more severe course and more rapid progression than those with AIH type 1/3 (19).

Overall, approximately 25% of patients show an acute onset of AIH, and rare cases of fulminant progression of AIH leading to acute liver failure have also been reported (20). Commonly, the clinical presentation of AIH resembles that of other forms of chronic hepatitis. AIH is therefore characterized by unspecific features, such as fatigue, right upper quadrant pain, jaundice, mild pruritus, arthralgias, and, less frequently, spider nevi and palmar erythema. In later stages, signs of portal hypertension including ascites, bleeding esophageal varices, and encephalopathy dominate.

Patients with the initial presentation of acute onset, as well as that of chronic hepatitis, often show histologic evidence of cirrhosis at the onset of symptoms, thereby implicating that subclinical disease may precede the onset of symptoms by a substantial amount of time. Up to 25% of patients initially show signs of decompensated liver cirrhosis (21). AIH, especially type 2, is associated with a wide variety of other autoimmune disorders, in particular autoimmune thyroiditis, rheumatoid arthritis, and diabetes mellitus (Table 31.2).

Diagnosis

The diagnosis of AIH is based on both (a) clinical, serologic, and immunologic features and (b) the exclusion of other hepatobiliary diseases with and without autoimmune phenomena, such as chronic hepatitis C, drug- or alcohol-induced hepatitis, nonalcoholic steatohepatitis (NASH), Wilson disease, genetic hemochromatosis, and α_1 antitrypsin deficiency. Cryptogenic hepatitis, which is an etiologically undefined chronic hepatitis, should also be included in the differential diagnosis of AIH. Patients with cryptogenic hepatitis are negative for viral and autoantibody markers. It is unclear how many of these patients suffer from AIH without detectable autoantibodies. If cholestatic signs and immunoserologic markers of AIH are present, overlap syndromes between AIH and primary biliary cirrhosis or primary sclerosing cholangitis have to be included in the differential diagnostic considerations. Liver histology by itself is not sufficient to prove the diagnosis of AIH but has a role in grading and staging of the disease. The revised AIH diagnostic score contributes to the establishment of the diagnosis in difficult cases by calculating a probability expressed as a numeric score (Table 31.3) (2).

Table 31.2. Extrahepatic Autoimmunologic Disease Associations of Autoimmune Hepatitis

Hematologic diseases	Autoimmune hemolytic anemia
	Thrombocytopenic purpura
	Pernicious anemia
	Eosinophilia
Gastrointestinal diseases	Inflammatory bowel disease[a]
	Celiac disease
Rheumatologic diseases	Synovitis[a]
	Rheumatoid arthritis
	CREST syndrome
	Systemic sclerosis
	Sjögren's syndrome
Endocrine diseases	Diabetes mellitus
	Autoimmune thyroid disease[a]
Others	Proliferative glomerulonephritis
	Lichen planus
	Vitiligo
	Nail dystrophy
	Alopecia
	Uveitis

	Erythema nodosum

[a]Most frequently observed.
CREST, calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.

Natural Course and Prognosis

The natural history and prognosis of AIH are largely defined by the inflammatory activity present at the onset of disease and by the presence or development of liver cirrhosis. A 5- to 10-fold increase of the levels of aminotransferases and 2-fold elevation of levels of gammaglobulins are associated with a mortality of 90% without treatment (22,23). With the presence of cirrhosis, mortality is 58% in 5 years. However, the presence of cirrhosis does not influence the initial treatment response or short-term outcome (21). The course of AIH is also significantly influenced by the HLA antigen profile of

the affected individual. The presence of HLA B8 is associated with severe inflammation at presentation and a higher likelihood of relapse after treatment (24,25). Individuals with HLA DR3 have a lower probability of reaching remission, have more frequent relapses, and more frequently require liver transplantation. The HLA DR4–positive subgroup is characterized by a higher age of onset and a more benign outcome (26,27).

Table 31.3. Revised Scoring System for Diagnosis of Autoimmune Hepatitis of the International Autoimmune Hepatitis Group (2)

Category	Score
Sex	
Female	+2
Male	0
Ratio of alkaline phosphatase to aminotransferase	
>3.0	-2

1.5–3.0	0
<1.5	+2
Elevation in the levels of (times upper limit of normal) of serum globulin (total), γ-globulin, or IgG	
>2	+3
1.5–2.0	+2
1.0–1.5	+1
<1.0	0
Autoantibodies (adults) ANA, SMA, or LKM-1	
>1:80	+3
1:80	+2
1:40	+1
<1:40	0
Antimitochondrial antibodies	
Positive	-4
Negative	0
Hepatitis viral markers	
Positive	-3
Negative	+3
History of drug usage	

Yes	-4
No	+1
Alcohol (average of consumption)	
<25 g/d	+2
>60 g/d	-2
Histology	
Interface hepatitis	+3
Predominant lymphoplasmacytic infiltrate	+1
Rosetting of liver cells	+1
None of these	-5
Biliary changes	-3
Other changes	-3
Seropositivity of other defined autoantibodies	
—	+2
Genetic factors	
HLA DR3 or HLA DR4	+1
Other autoimmune diseases	
—	+2
Response to therapy	
Complete	+2

Relapse		+3

Interpretation of aggregate scores:		
Pretreatment	Definite AIH	>15
	Probable AIH	10–15
Posttreatment	Definite AIH	>17
	Probable AIH	12–17.

IgG, immunoglobulin G; ANA, antinuclear antibody, SMA, smooth muscle antibody, LKM, liver–kidney microsome; HLA, human leukocyte antigen; AIH, autoimmune hepatitis.

The presence of autoantibodies does not generally correlate with disease activity, disease progression, or treatment outcome. However, in some cases autoantibodies may be of prognostic value. In AIH type 1,

antibodies against actin identify a subgroup of anti-SMAs–positive patients with earlier onset of disease and a higher frequency of refractoriness to steroid treatment (28). The presence of anti–asialoglycoprotein receptor (anti-ASGPR) autoantibodies in patients with AIH type 1 is associated with increased laboratory and histologic disease activity and more frequent relapses after steroid withdrawal (29,30).

In case of AIH type 2, autoantibodies to liver cytosol type 1 (anti-LC1) appear to correlate with disease activity, whereas LKM autoantibodies do not (31).

Treatment Indications

AIH was the first chronic liver disease in which medical treatment was proved to prolong survival. A sustained serum aspartate aminotransferase (AST) abnormality of at least 10-fold of normal or a serum AST level of at least 5-fold of normal in combination with serum gammaglobulin level at least twice of normal represents an absolute treatment indication. Histologic evidence of bridging necrosis or multilobular necrosis, as well as severe hepatic and extrahepatic symptoms, also represents absolute treatment indications.

Mild clinical symptoms and less severe laboratory or histologic abnormalities are relative treatment indications. The benefit of immunosuppressive treatment of patients with mild symptoms remains unclear because further reduction of the already low incidence of cirrhosis and of long-term mortality may not occur at acceptable risk.

No treatment indications exist for patients with inactive cirrhosis, complications of portal hypertension in the absence of hepatitis, or mild interface hepatitis in the

absence of symptoms (32).

Induction of Remission

The standard initial treatment of AIH is either prednisone or prednisolone monotherapy (40 to 60 mg/day and tapering regimen) or combination therapy with prednisone (20 to 30 mg/day and tapering regimen) and azathioprine (1 to 2 mg/kg body weight per day) (33,34,35,36). Although steroid monotherapy is as effective as the combination therapy with azathioprine in inducing remission, azathioprine is ineffective in the induction of remission on its own, and this should not be attempted. Combination therapy is generally preferred because it allows for the reduction of the prednisone dose frequently to below 10 mg/day, thereby reducing the steroid-associated side effects. Conversely, candidates for the sole use of prednisone are patients with severe cytopenia or active malignancy or women who are pregnant or planning pregnancy, although the teratogenicity of azathioprine in humans remains a controversial issue.

Conventional treatment is continued until remission, treatment failure, drug toxicity, or incomplete response results. Remission is defined as a complete biochemical and histologic resolution of inflammation, as well as the disappearance of clinical symptoms. Histologic remission lags 6 to 12 months behind normalization of biochemical markers, and prompt discontinuation of medication without histologic documentation of resolution might result in an exacerbation of the disease. Therefore, biopsy-proved remission is the ultimate goal of all treatment regimens.

Maintenance of Remission

If histologic remission is achieved, the risk of relapse is only approximately 20% (35). In contrast, if biopsy specimens still show portal hepatitis, relapse occurs in 50% or more of patients within 6 months of the end of treatment. Relapse is characterized by deterioration of clinical signs and increase of serum aminotransferase levels after remission and drug withdrawal. Overall, a sustained response is achieved only in 17% of patients after withdrawal of immunosuppressive therapy. It has been demonstrated that azathioprine maintenance after prednisone withdrawal reduces the likelihood of relapse (37,38). Therefore, maintenance of immunosuppressive therapy with azathioprine (2 mg/kg body weight per day) alone or in combination with prednisone is generally recommended for at least 2 years. Prednisone withdrawal should proceed gradually over a period of 3 to 6 months.

Failure of Remission

Treatment failure is characterized by the deterioration of biochemical and clinical signs during therapy, whereas patients with an incomplete response show clinical, laboratory, and histologic improvement, but all manifestations of their disease are not resolved. One reason for treatment failure might be the intolerance to the administered drug in a certain proportion of patients. In these situations standard treatment schedules have to be altered or alternative drugs considered. In about 10% to 15% of patients the standard therapy failed, although it was well tolerated. Whether these patients show a different genetic background in regard to the pathogenesis of disease or drug metabolisms remains unclear. Nevertheless, an underlying overlap syndrome should be excluded.

Alternative Treatment Strategies

In addition to standard therapy, new approaches using various immunosuppressive agents have been tested (Table 31.4). These have emerged mainly from the field of transplantation and should be considered if standard treatment fails.

Cyclosporin A (3 to 5 mg/kg body weight per day) binds to cyclophilin and inhibits calcineurin action. This results in the downregulation of interleukin-2 (IL-2) and T-cell function. Several studies in a small number of patients demonstrated a benefit from cyclosporin A treatment in both AIH type 1 and type 2 (39,40,41,42). However, cyclosporin A has not been compared with standard treatment in a randomized manner. Its toxicity profile, particularly with long-term treatment, limits the widespread use of this drug. Among the most frequently observed side effects are renal failure and hypertension.

Tacrolimus (3 mg twice daily) binds to FK-binding protein 12 (FKBP12). The complex inhibits calcineurin, thereby inhibiting the synthesis of cytokines and the expression of the IL-2 receptor that suppresses T-cell function. Preliminary trials showed a significant biochemical improvement in patients treated with high-dose tacrolimus alone or with lower doses in combination with glucocorticoids (43,44). The side effects resemble those of cyclosporin A and may limit the long-term use of the drug.

Table 31.4. Current Options in the Treatment of Autoimmune Hepatitis

Drug	Dosage	Actions	Side effects
Prednisone	40–60 mg/d and tapering regimen	Inhibition of proinflammatory transcription factors/cytokines	Cushing's syndrome Osteopenia Diabetes mellitus Glaucoma Hypertension
Azathioprine	1–2 mg/kg BW per d	Inhibition of nucleic acid synthesis	Leukopenia/anemia Pancreatitis Nausea
Cyclosporin A	3–5 mg/kg BW per d	Downregulation of IL-2 and cytotoxic T-cell function	Hypertension Renal failure
Tacrolimus	3 mg twice daily	Inhibition of IL-2/IL-3/IFN-γ, of IL-2-receptor expression, and of cytotoxic T-cell function	Hypertension Renal failure Diabetes mellitus Pancytopenia
Mycophenolate	1,000	Inhibition of DNA	Nausea/diarrhea

mofetil	mg twice daily	synthesis and B-/T-cell proliferation	Leukopenia
Budesonide	3 mg thrice daily	Local inhibition of transcription factors/cytokines (high first-pass metabolism)	In the absence of cirrhosis or portosystemic shunts, side effects are rare and resemble those of systemic steroids
Deflazacort	7.5 mg daily	Inhibition of proinflammatory transcription factors/cytokines	Fewer side effects than other systemic steroids
Cyclophosphamide	1–1.5 mg/kg BW per d	Alkylating/DNA-damaging agent	Pancytopenia Cystitis

BW, body weight; IL, interleukin; IFN, interferon; DNA, deoxyribonucleic acid.

Mycophenolate mofetil (1 g twice daily) is an ester prodrug of mycophenolic acid that inhibits inosine monophosphate dehydrogenase, resulting in the depletion of guanine nucleotides and inhibiting deoxyribonucleic acid (DNA) synthesis. Preliminary data suggest that mycophenolate mofetil alone or together with prednisolone may represent an alternative treatment strategy in the induction or maintenance of remission, especially for those patients intolerant of or resistant to azathioprine therapy (45,46). Gastrointestinal discomfort is the main side effect. Myfortic, a novel immunosuppressive drug related to mycophenolate, may help overcome this problem.

Budesonide (3 mg three times daily) is a second-generation glucocorticoid with a high first-pass clearance by the liver. It is therefore characterized by reduced systemic side effects compared to conventional steroids. In a small study

including 13 patients, a beneficial effect of budesonide treatment over a period of 9 months could be demonstrated (47). Other investigators have questioned the benefit of budesonide (48). Remission can be achieved when budesonide is used instead of prednisolone (49). However, the main advantage of budesonide for the future treatment of AIH is to replace prednisolone in long-term maintenance therapy to reduce side effects of the steroid. Larger studies are under way to prove this potential benefit.

Deflazacort (7.5 mg daily) is an oxazolinic derivative of prednisolone with fewer

effects on bone and glucose metabolism than the parent molecule. In a small study including 15 patients, deflazacort was able to maintain remission. However, the efficacy and tolerance of deflazacort have not been tested in large cohorts of patients and long-term trials (50).

Cyclophosphamide (1 to 1.5 mg/kg body weight per day) is an alkylating chemotherapeutic drug that leads to DNA damage. In small case studies, cyclophosphamide in combination with corticosteroids was able to induce and maintain remission (51). However, because of its potentially severe hematologic side effects, continued application of cyclophosphamide remains a highly experimental treatment option.

Methotrexate (7.5 mg/week) is an antimetabolite with both immunosuppressant and antiproliferative effects. There is a single case report of successful induction and maintenance of remission of a patient with AIH type 1 refractory to standard therapy (52).

6-Mercaptopurine (1.5 mg/kg body weight per day) is an active metabolite of azathioprine. The purine analog inhibits T-cell proliferation by interfering with nucleic acid metabolism. 6-Mercaptopurine in combination with corticosteroids was demonstrated to be effective in the induction and maintenance of remission in three patients with AIH who either could not tolerate or failed to improve with azathioprine. 6-Mercaptopurine allows for the reduction of the prednisone dosage to or below 10 mg/day, thereby reducing the steroid-associated side effects (53).

Liver Transplantation

For patients refractory to medical treatment who progress to cirrhosis or do not reach remission within 4 years of continuous therapy, liver transplantation remains the treatment option of choice. Favorable 10-year survival rates of approximately 75% to 85% have been reported (54). However, recurrence of AIH is estimated to range between 11% and 41% (55,56,57,58,59,60). Persistence of autoantibodies does not indicate or predict recurrence of AIH (61). Moreover, patients undergoing transplantation for AIH might have a higher frequency of acute, chronic, and steroid-resistant rejection compared to those undergoing transplantation for other diseases (62,63). Careful monitoring of the patients with AIH who have undergone transplantation and individual adjustment of immunosuppressive therapy after transplantation are therefore necessary to prevent or control recurrence of AIH.

A distinct disease entity is de novo AIH after liver transplantation in patients undergoing the procedure for other causes of end-stage liver disease (64,65). The incidence of this type of AIH appears to be higher in children than in adults. A possible mechanism leading to de novo AIH after transplantation is a deranged elimination of autoreactive T-cell clones because of immunosuppression.

Further identification of the underlying molecular mechanisms of AIH recurrence, de novo AIH, and allograft rejection is necessary to clearly distinguish between these immunologically distinct conditions and to develop site-specific therapies.

Future Directions and Conclusions

Increasing insights into the molecular mechanisms of AIH will open new strategies for the development of novel immunosuppressive agents. Drugs have been established that disrupt the intracellular signaling pathways involved in AIH such as apoptosis or transcription of proinflammatory cytokines. In this respect, FTY-720, a novel immunosuppressive agent that exerts its effect through induction of lymphocyte apoptosis, may be a promising candidate immunosuppressant (66). Cytokine interactions can be manipulated by administering recombinant species or

be inhibited by the use of monoclonal antibodies. Human recombinant IL-10 and monoclonal antibody against TNF-α have each been tested in clinical trials (67,68,69,70,71,72). Targeting of various cytokines might therefore be effective in downregulating the immune response in AIH. T-cell vaccination that eliminates disease-specific T-cell clones might be another promising strategy because in an experimental AIH model it has been shown to not only prevent disease but also decrease disease activity as effectively as steroids (73). Similarly, antibodies against the protein product of the T-cell gene (TIRC7) that inhibits T-cell proliferation might be a future therapy (74). Agents that block transendothelial migration of effector T cells into target tissues are also feasible (75,76). Synthetic peptides that compete with self-antigen or foreign antigen that resembles self-antigen can inhibit the first costimulatory signal of immunocyte activation by occupying the binding grooves of the class II molecules of the major histocompatibility complex. Therapy with synthetic peptides is already being evaluated in rheumatoid arthritis (75,76). Soluble CTLA-4 that competes with CD4 helper T cells for interaction with APCs could also downregulate autoimmune response (77,78). Finally, gene therapy may represent a further strategy in the future that is capable of counterbalancing the overproduction of proinflammatory cytokines and the progression to fibrosis in addition to promoting liver regeneration in the course of AIH (79).

In summary, the therapeutic goal of the novel immunosuppressive agents includes the achievement of long-term remission in patients who do not respond to or do not tolerate standard therapy. However, long-term side effects of the novel immunosuppressants are a matter of concern. Larger studies are required to

provide more evidence for their risks and benefits that would justify their use as an alternative strategy to standard therapy.

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Chapter 32

Alcoholic Liver Disease

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Arthur J. Mccullough

Key Concepts

- Although 90% to 100% of heavy drinkers show evidence of fatty livers, only 10% to 35% develop alcoholic hepatitis and 5% to 15% develop cirrhosis.
- The daily intake of alcohol that results in liver injury varies and depends on a number of modifiable and unmodifiable factors. Alcoholic liver disease (ALD) develops at much lower doses, especially in women, Hispanics, and patients with hepatitis C infection.
- Insights into the pathogenesis of alcohol-induced liver injury have improved significantly, but translation into clinical benefit has been slow. Interaction among the products of alcohol metabolism, hepatic parenchymal and nonparenchymal cells, and chemokine release all contribute to the injury and progression of the disease.
- The importance of continued abstinence and correction of nutritional deficiencies are major components in the long-term management of ALD.
- Alcoholic hepatitis has a variable mortality and the prognosis is determined most commonly by the modified discriminant function. Further studies are required to establish the prognostic accuracy of the Model for End-Stage Liver Disease (MELD) or the recently described Glasgow alcoholic hepatitis score (GAHS).
- Anti-inflammatory therapy with corticosteroids and anticytokine therapy with pentoxifylline are effective and are evidence-based therapy for patients with severe alcoholic hepatitis. It may be necessary to perform a liver biopsy in patients with ALD because the clinical diagnosis is not always accurate. This is of particular relevance when corticosteroids are being considered for treating alcoholic hepatitis.
- Patients with end-stage ALD should be considered for liver transplantation. Six months of abstinence is usually required before transplantation, but this length of time may be adjusted on an individual basis.
- Liver transplantation is being increasingly offered to patients with alcoholic cirrhosis and the results are similar to those in nonalcoholic patients. Post-transplantation alcohol abuse is a clinical issue that needs to be addressed throughout the pre- and post-transplantation setting. The role of liver transplantation, especially using living donors, for acute alcoholic hepatitis is a matter of debate.

Table 32.1. Characteristics of Alcoholism

Characteristic	Clinical feature
1. Tolerance	A state of adaptation in which increasing amounts of alcohol are needed to produce the desired effects
2. Physical dependence	A typical withdrawal syndrome appears on interruption of drinking, which is relieved by alcohol itself or other drugs in the alcohol/sedative group

3. Impaired control	Total alcohol intake cannot invariably be regulated once drinking has begun at any drinking occasion
4. Craving	A dysphoria of abstinence that leads to relapse
The Acronym TyPICal is suggested as an aid to clinicians to diagnose alcoholism.	

Worldwide, alcohol is the most frequently used and socially acceptable hepatotoxin (1). Geographic patterns of alcohol intake and the prevalence of alcoholic liver disease (ALD) are changing constantly with the rapid increase in per capita alcohol ingestion in eastern European countries and stabilized use in western European countries, Canada, and Australia (2). Approximately two thirds of the adult Americans drink some alcohol (3). Most drink light or moderate amounts and do so without problems (4,5,6,7); however, a subgroup of drinkers become dependent on alcohol and have the disease of alcoholism (8,9). Another group of drinkers are alcohol abusers (and problem drinkers) who experience negative consequences of drinking (e.g., unemployment, loss of family, or accidental injury or death). These patients are not considered to be alcohol dependent (7,10). Failure to recognize alcoholism remains a significant problem that impairs both the prevention and management of ALD (10,11). The clinical features of *t*olerance, *p*hysical dependence, *i*mpaired control, and *c*raving that define *al*coholism (shown in Table 32.1), as well as their acronym TyPICaL, are suggested as aids to the clinician for making the diagnosis. For more complete and formal diagnostic criteria of alcohol-use disorders, publications by various organizations including the American Psychiatric Association and the World Health Organization are recommended (12,13).

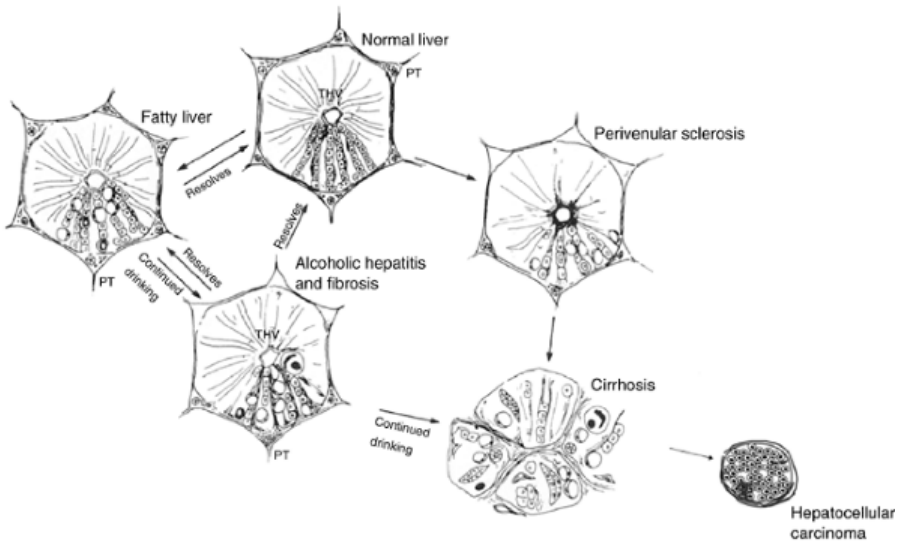
Disease Spectrum

Alcohol affects the liver depending on the dose and the duration of use or abuse (14,15,16). The spectrum of alcohol-related liver injury varies from asymptomatic hepatomegaly to profound hepatocellular failure and portal hypertension (17,18,19). Pathologically, this is translated into fatty infiltration of the hepatocytes at the stage of transition of asymptomatic hepatomegaly to frank cirrhosis, with decompensation at the end stage (17,20,21). There are at least five histologic manifestations of ALD, which include fatty liver or steatosis, acute alcoholic hepatitis, chronic hepatitis, hepatic fibrosis, and cirrhosis (22). Of these, chronic hepatitis is a stage that has been reported in ALD, but its diagnosis has been questioned. (23,24).

Figure 32.1 displays the different stages and evolution of ALD, and Table 32.2 lists the histologic characteristics and describes their prevalence in the different stages of ALD (22). Fatty liver develops in virtually every individual who drinks more than 60 g/day of alcohol and initially localizes to the perivenular or centrilobular region of the liver (25,26,27). Decreased energy stores due to hypoxia and a shift in lipid metabolism along with a shift in the redox reactions caused by the preferential oxidation of alcohol in zone 3 of the hepatic lobule are the reasons for this localization (Fig. 32.2) (27,28). Simple, uncomplicated fatty liver is usually asymptomatic and considered reversible (29,30). Once fatty infiltration becomes severe, particularly with a mixed (macro-/microvesicular) pattern and associated with giant mitochondria or perivenular fibrosis (26,31), progression to fibrosis and cirrhosis (8% to 20% of patients) can occur.

Progression of ALD culminates in scarring and development of cirrhosis (32). Although usually micronodular, it is occasionally mixed micro- and macronodular (19,33). Fibrogenesis is believed to start in the perivenular area and is influenced by the amount of alcohol ingested (26,34). Perivenular fibrosis and deposition of fibronectin occurs in 40% to 60% of patients who ingest more than 40 to 80 g daily for an average of 25 years. However, the thickness of the perivenular fibrosis does not correlate with the amount of alcohol ingested. It should also be noted that the occurrence of the fibrotic features was more than twice the frequency of cirrhosis. Although this may be partly related to sampling error, the disparity between the frequency of fibrosis and the development of cirrhosis suggests that factors other than fibrogenesis are involved in the progression of fibrosis to cirrhosis in patients with ALD (35).

Figure 32.3 shows the estimated prevalence of the different histologic forms of alcohol-mediated liver injury among heavy drinkers (22). This emphasizes the heterogeneity of the patient populations with regard to disease severity and individual susceptibility to alcohol. Among patients who are heavy drinkers, 90% to 100% will show histologic evidence of fatty liver but only 10% to 35% will develop alcoholic hepatitis and 8% to 20% will develop cirrhosis (36,37).



▪ **Figure 32.1** The schematic form of the progression and potential regression among the various histologic stages of alcoholic liver disease. *THV*, terminal hepatic venule; *PT*, portal tract.

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Risk Factors

Only a minority of individuals who ingest significant amounts of alcohol progress beyond fatty liver and develop alcoholic hepatitis or cirrhosis. Therefore, other factors must play a role in placing these individuals at risk for developing these more severe forms of ALD (30,38). A number of risk factors have been proposed (Fig. 32.4), but none of them can either singly or in combination completely explain the reason why only a minority of individuals ingesting large amounts of alcohol develop ALD (39,40).

Ethnicity

Ethnic differences in the prevalence of alcohol-related liver disease and associated mortality have changed with time (2). In the first half of the 20th century, when reporting of mortality was done for whites and nonwhites (composed predominantly of African Americans), the mortality was significantly higher for the former. By the mid-1950s, this reversed and the mortality for nonwhites exceeded that for whites. Later, when data was published for different ethnic groups, a twofold increase in the level of transaminases was found to occur more frequently in Hispanics and Blacks than

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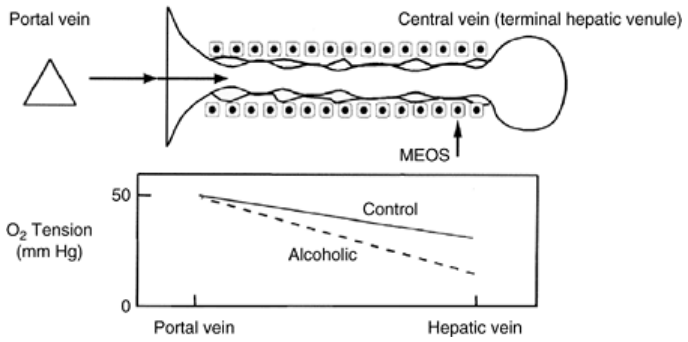
in whites (41). Recently, South Asian (Indian) non-Muslim men have been observed to have a higher rate of alcoholic cirrhosis at a younger age and after a shorter duration of alcohol abuse (42). It is not clear whether the ethnic differences are the result of genetic polymorphisms, or quantity or type of alcoholic beverages consumed (2).

Table 32.2. Histologic Characteristics of Alcoholic Liver Disease

	Fatty liver	Alcoholic	Cirrhosis	Cirrhosis–alcoholic

	(%)	hepatitis (%)	(%)	hepatitis (%)
Ballooning degeneration with PMNs	73	97	76	35
Mallory bodies	0	76	19	95
Megamitochondria	100	32	8	13
Sclerosing hyaline necrosis	4	68	3	44
Fibrosis	31	54	100	100
Fat (moderate to severe)	69	82	27	43
Perivenular fibrosis	4.9	19	—	—

PMN, polymorphonuclear cells.
These data were obtained and modified from MacSween RN, Burt AD. Histologic spectrum of alcoholic liver disease. *Semin Liver Dis* 1986;6(3):221–232.



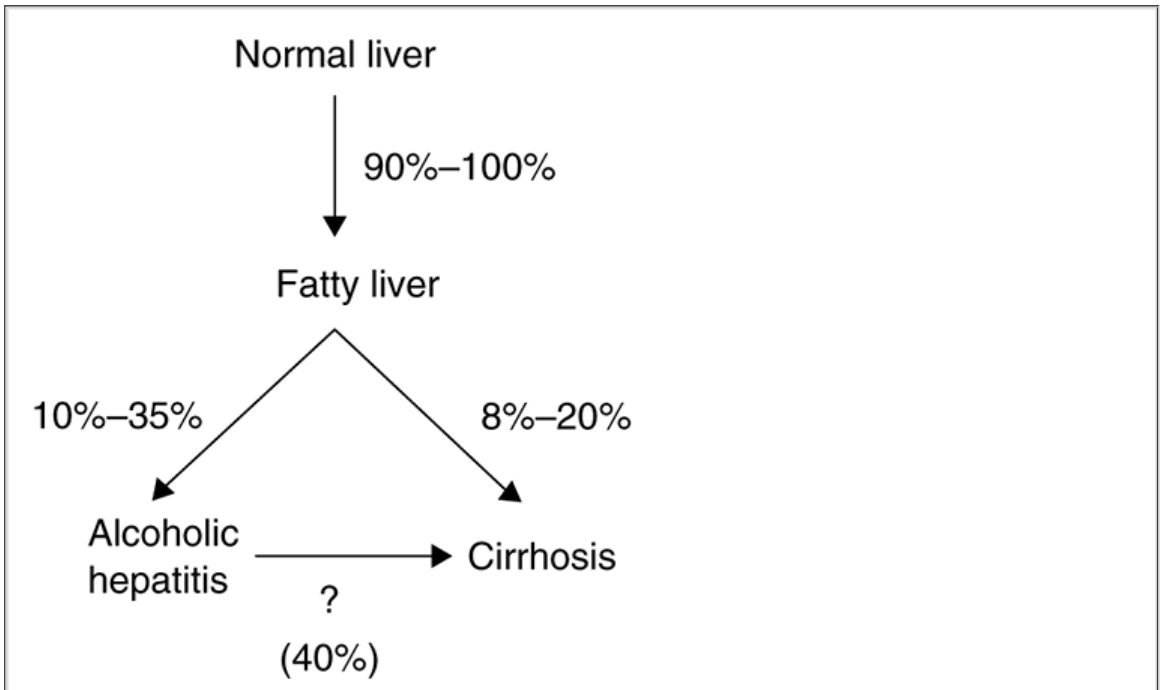
▪ **Figure 32.2** The oxygen gradient between the portal area (zone 1) and the pericentral area (zone 3) of the hepatic lobule. The metabolism of alcohol increases oxygen consumption and causes the largest gradient in zone 3. MEOS, microsomal ethanol-oxidizing system.

Alcohol

The quantity of alcohol ingested (independent from the form in which it is ingested) is an important risk factor for the development of ALD (43). A significant correlation exists between per capita consumption and the prevalence of cirrhosis. Epidemiologic evidence exists for a marked reduction in the prevalence of ALD, with diminished alcohol ingestion during war rationing, prohibition, and increased cost. (44,45).

Available evidence indicates that there is an increased risk of developing cirrhosis with the ingestion of more than 60 to 80 g/day of alcohol in men and more than 20 g/day in women, when consumed for 10 years or longer (2,16,46). Even in these groups, only 6% to 41% of those drinking at such levels develop cirrhosis (2,16,47). In the Dionysos study, even in patients with

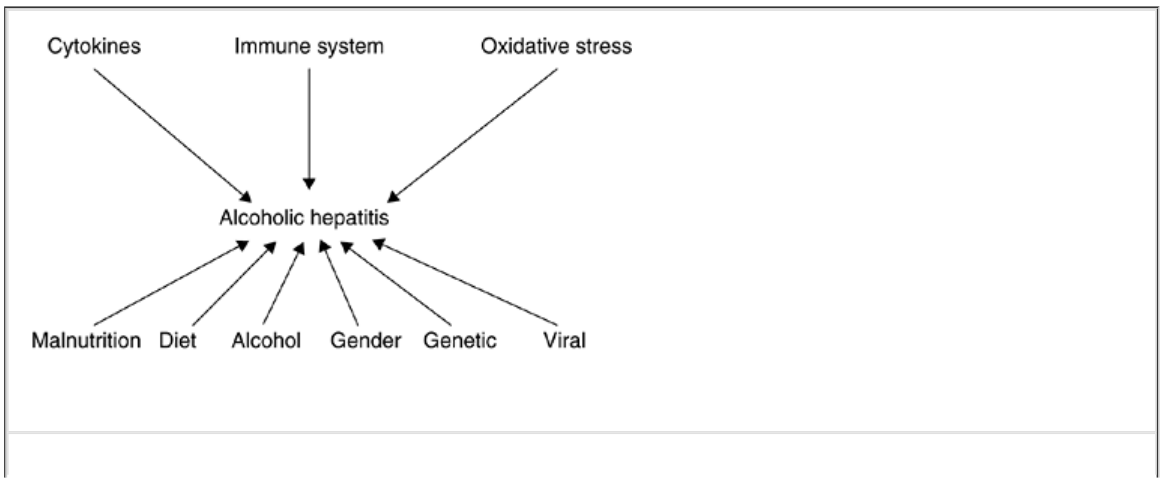
very high daily alcohol intake (>120 g/day) only 13.5% developed alcohol-induced liver damage (46,48). In addition, disagreement exists on whether the amount of alcohol consumption increases the risk of ALD proportionally. However, population-based data consistently confirm that the quantity of alcohol ingested is a risk factor for ALD (49). In the Dionysos study, the risk of cirrhosis or noncirrhotic chronic liver disease increased with a total lifetime alcohol intake of more than 100 kg or a daily intake of more than 30 g/day (46). The odds of developing cirrhosis or noncirrhotic liver disease with a daily alcohol intake of more than 30 g/day was 13.7 and 23.6, respectively, when compared with nondrinkers.



▪ **Figure 32.3** A schematic displaying the percentage of heavy drinkers who develop the different stages of alcoholic liver disease. ?, uncertainty regarding the precise percentage but may be as high as 40%.

There is now increasing evidence that the type of alcohol also determines the risk of development of liver disease (50). In a larger survey of over 30,000 persons in Denmark, beer or spirits were found more likely to be associated with liver disease. It was also observed that in persons who drank heavily, consumption of wine lowered the all-cause mortality (51,52). It is however

unclear whether wine drinking was itself protective or was a surrogate for other healthy behaviors such as consumption of fruits and vegetables (52). Alcoholic beverages that contain a higher content of short-chain aliphatic alcohols have been associated with a high prevalence of liver cirrhosis (53).



▪ **Figure 32.4** The risk factors, which may be cofactors required for the development of advanced alcoholic liver disease.

Another factor that has been identified is the contribution of the pattern of drinking to the development of liver injury. Drinking outside of mealtime has been reported to increase the risk of ALD by 2.7-fold compared to those who consumed alcohol only at mealtime (54). This was not, however, reproduced in a subsequent French study (55). Binge drinking, which may be considered to be a form of outside mealtime drinking, has also been shown to increase the risk of ALD and all-cause mortality (56). Binge drinking has also been shown to be associated with degradation of a large quantity of mitochondrial deoxyribonucleic acid (DNA) that contributes to steatohepatitis and reperfusion injury, both of which result in significant damage to the hepatocytes and potentially nonparenchymal hepatic cells (57).

Gender

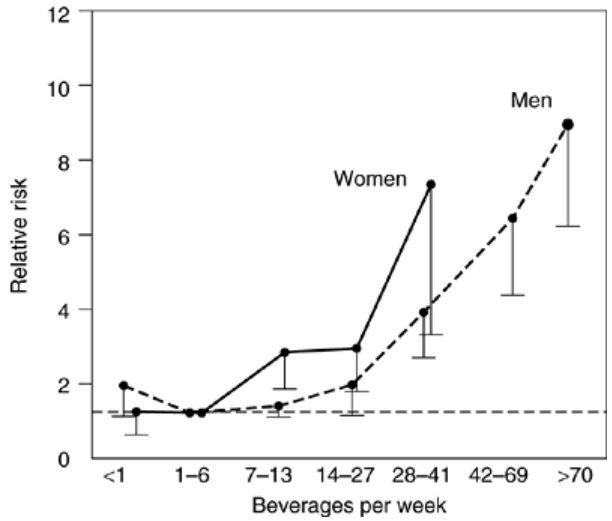
Women have been found to be twice as sensitive to alcohol and develop more severe ALD at lower doses and with shorter duration of alcohol consumption than men (27,58,59,60,61). As compared to men, in whom 80 g of daily alcohol consumption was considered to be a hazardous amount, early data suggested the hazardous level to be 60 g daily in women (62). Because most of those individuals developing ALD ingested more than 35 units/week, a "safe" limit of alcohol intake had been suggested to be 21 units/week in men and 14 units/week in women (62,63). However, more recent data from the Copenhagen City study suggest that a lower quantity, more than 7 units/week, may be toxic in women (49,64). The data in Table 32.3 and Figure 32.5 confirm the association between increased alcohol intake and ALD, the lower threshold toxic dose, and the increased female susceptibility for ALD. On a practical level 1 unit of alcohol (1 ounce of "spirits" [40% alcohol], one 12-ounce beer [5% alcohol], or one 4-ounce glass of wine [12.5% alcohol]) contains approximately 10 g of alcohol for wine and spirits and 14.4 g of alcohol for beer. This is based on a specific gravity of 0.8 for alcohol and the average alcohol content of beer. These amounts may vary depending on the actual alcohol content of beer, which varies significantly among commercial brands (Table 32.4), as well as the size of the pour for wine and spirits.

Table 32.3. Relative Risk of Alcoholic Liver Disease at Different Levels of Alcohol Intake

	Alcoholic cirrhosis		Alcoholic liver disease	
Weekly units of alcohol	**Men**	**Women**	**Men**	**Women**
<1	3.7	1.09	1.8	1.0
1–6	1.0	1.0	1.0	1.0
7–13	0.9	4.1[a]	1.1	2.9[a]
14–27	1.6	3.1[a]	1.4	2.9[a]
28–41	7.0[a]	16.8[a]	3.8[a]	7.3[a]
42–69	13.0[a]	NR	5.9[a]	NR
≥70	18.1[a]	NR	9.1	NR

These results were estimated from Figure 1 in reference 49.
[a]Represents a statistically significant increased relative risk of having alcoholic liver

disease.
NR, not reported.



▪ **Figure 32.5** Risk of alcoholic liver injury increases with increasing quantity of alcohol intake. This risk is higher in women at all doses of alcohol ingestion.

This increased female susceptibility has been related to gender-dependent differences in the hepatic metabolism of alcohol in the past (27,65). Alteration in gastric metabolism of alcohol is another explanation for the female susceptibility to develop ALD (58,65,66,67). First-pass gastric metabolism of alcohol does indeed exist in humans (65), but the gastric metabolism of alcohol may not be large (10% to 30% at most), decreases with gastric injury, may not occur during

most standard meals or realistic life conditions, and may vary among races because it has not been observed in Orientals (68,69). Furthermore, the lack of increased risk of ALD in postgastrectomy patients has also refuted this hypothesis (70). Experimental evidence exists for estrogen-induced endotoxemia and exaggerated hepatic inflammatory response to alcohol (71,72). The clinical significance of these proposed mechanisms is as yet unclear.

Table 32.4. Alcohol Content (%) of Commercial Beer

Brand	Light	Regular
Molson	2.41	3.88
Bud	3.88	4.82

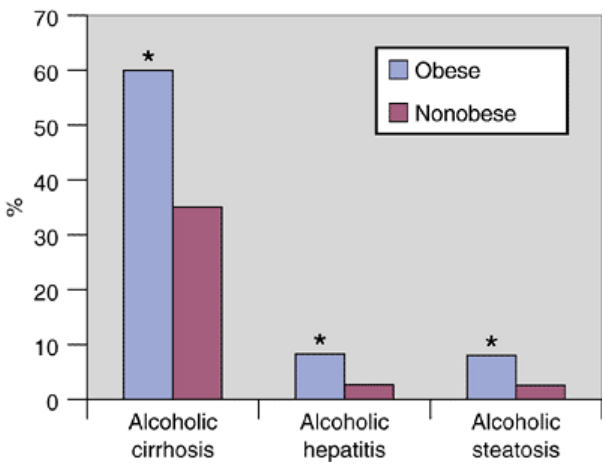
Busch	4.12	5.19
Miller	4.40	4.80
Stroh's	4.45	4.68
Michelob	4.52	4.99

Malnutrition and Diet

Dietary habits and nutritional status may also be important risk factors (16,18,73,74,75). Early studies conducted in hospitalized chronic alcoholic patients with liver disease led to the misconception that protein calorie malnutrition was a necessary risk factor for ALD (75,76). However, alcohol is directly hepatotoxic and does not require preexisting malnutrition to result in liver injury (77,78). In addition, although alcohol adversely affects energy and protein metabolism, the prevalence and extent of protein calorie malnutrition are similar in alcoholic and nonalcoholic cirrhosis (79).

Micronutrient abnormalities also potentially aggravate the liver disease. Hepatic vitamin A depletion activates the stellate cells and results in hepatic fibrosis with alcohol abuse. Vitamin E levels are depressed in alcoholic cirrhosis and may contribute to the enhanced membrane lipid peroxidation and hepatocyte damage (80).

Other nutritional disorders including obesity and dietary habits may be important risk factors (16,81,82). Data from France (Fig. 32.6) suggest that obesity may be an independent risk factor for developing ALD (82). In alcoholic patients from China, excess body weight was associated with a 5.6-fold increase in the risk for ALD (54). Increased tumor necrosis factor (TNF) activity and hepatic insulin resistance that are associated with obesity seem to contribute to the aggravation of ALD (83). Obesity also makes the liver susceptible to alcohol-mediated injury by metabolic activation of CYP 2E1, oxidant stress, and immune hyperreactivity in the liver (84). It is currently unclear whether the hepatotoxic consequences of obesity and ethanol ingestion are additive or synergistic. High-fat diets are also necessary to promote alcohol-induced liver disease in animals (Fig. 32.7) (85,86). In addition, the incidence of cirrhosis appears to be lower than that expected in countries with high intakes of saturated fat; an epidemiologic finding that is independent of other risk factors and supported by animal data (87,88).



▪ **Figure 32.6** A histogram showing the prevalence of obesity in different forms of alcoholic liver disease in obese patients. *, *P* <.05 between groups. (Extracted and modified from Naveau S, Giraud V, Borotto E, et al. Excess weight risk factor for alcoholic liver disease.

Hepatology 1997;25(1):108–111.)

Genetic

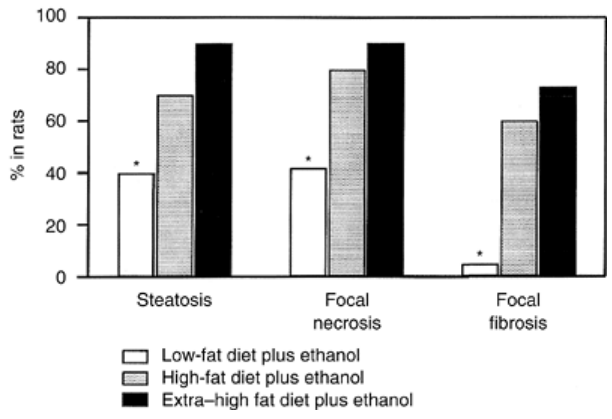
Environmental factors alone cannot explain variable susceptibility to alcohol, and genetic factors appear to provide a predisposition to both alcoholism and ALD (89,90,91,92). There has been growing interest in defining genetic factors that include functional polymorphisms in genes that may contribute to the pathophysiology of ALD (93,94,95).

Alcohol metabolism

Much of the initial work focused on the genes involved in the metabolism of alcohol: Alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH), and the cytochrome P-450 system (CYP 4502E1) (96,97,98,99). The most commonly reported abnormality is an increased frequency of ADH3-1 (gene that encodes for the γ-1ADH isoenzyme capable of faster metabolism of alcohol to acetaldehyde) that when combined with abnormalities in the ALDH2-2 allele (slow acetaldehyde metabolism) results in high acetaldehyde concentration (97,100,101). Such an abnormality may be responsible for some of the hepatotoxicity of alcohol and mediates the flushing and enhanced alcohol sensitivity commonly observed in Asians (102,103,104). Patients in Japan have a higher frequency of ADH2-1, and an increased frequency ALDH2-1 has been reported in patients who are alcoholics (with and without liver disease) than in controls (105). However, the data on a specific ADH/ALDH pattern in patients with ALD are conflicting.

The genetic pattern of P4502E1 (the microsomal enzyme primarily involved in alcohol metabolism) has been studied because it generates reactive oxygen species (ROS) when metabolizing alcohol (104,106). A specific polymorphism identified as the c2 allele has been shown to result in an increased expression of hepatic CYP 2E1 activity. An increased prevalence of the c2 allele has been reported in Japanese but not Chinese

alcoholic subjects with ALD (105,107,108). A higher prevalence of c2 in Mexican Americans than whites has also been reported (101). However, a meta-analysis of nine independent studies has shown that there was no relation between the c2 allele and the development of ALD among whites (99).



▪ **Figure 32.7** A histogram showing the effect of high-fat diet on the severity of liver disease along with alcohol feeding in experimental animals. *, *P* <.05 versus other two groups.

Mitochondrial dysfunction

Mitochondrial dysfunction has been identified in the liver in ALD, and a reduced activity of the mitochondrial form of superoxide dismutase 2 (SOD2) was expected to have a reduced activity. In French patients with ALD, a polymorphism in the gene with a valine to alanine mutation at position 1183 of the SOD2 gene was observed, but this resulted, unexpectedly, in an increase in SOD2 activity (109). In a subsequent study from England, no association was observed between SOD2

polymorphism and ALD (110).

Cytokines and the immune system

Data supporting a role for endotoxin-mediated release of cytokines in the pathogenesis of ALD have suggested a different set of candidate genes with a potential role in disease susceptibility (111,112,113). Small studies have reported an association between advanced ALD and promoter polymorphisms in the genes encoding the endotoxin receptor CD14 and interleukin-10 (IL-10), which need further confirmation (114,115,116,117).

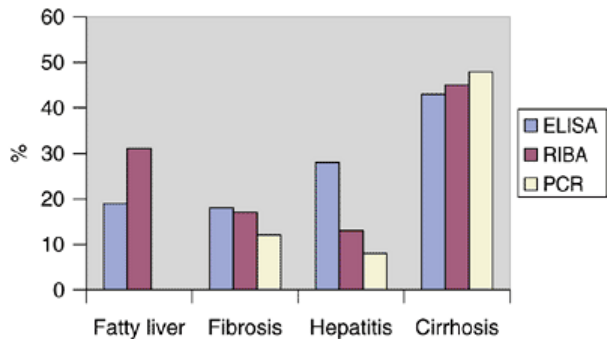
These abnormalities suggested an upregulation of immune response in these patients (118,119). Reported targets of this immune response are autoantigens, which include ADH, cytochrome P-450 isoforms CYP 2E1/CYP 3AH, and endogenous protein adducts to ethanol metabolites or products of lipid peroxidation (120,121). Polymorphisms in TNF-α (associated with alcoholic hepatitis), type I collagen (associated with cirrhosis) genes, and the gene encoding cytotoxic T lymphocyte–associated antigen-4 (CTLA-4) that functions as a suppressor of T cell–mediated immune response in patients with ALD have been reported (119,122,123,124,125,126,127). Studies from England and Spain have reported polymorphisms in the gene encoding IL-10, a cytokine that downregulates inflammatory response, resulting in lower levels of IL-10 in patients with ALD (123,128).

An integrated view of genetic polymorphisms and the consequent immune-mediated liver injury has been suggested by the observations that CTLA-4 gene polymorphism and lower activity were associated with an increase in antibodies to the hydroxyethyl CYP 2E1 adducts and the development of liver disease (127,129). Despite interesting and valuable new information in this area, there is variability in the reported association between genetic and phenotypic abnormalities in different studies and racial populations. Specific genetic abnormalities for a susceptibility to alcohol abuse and the development of ALD have not yet been firmly established.

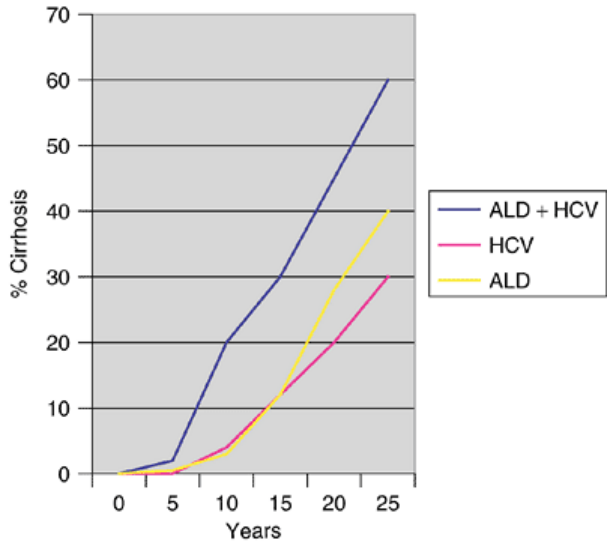
Viral Infection

There is a strong association between hepatitis C and ALD (130). Unlike hepatitis B, hepatitis C infection appears to be strongly involved in the development of advanced ALD (131). An increased prevalence of hepatitis C virus (HCV) has been reported in patients with

ALD (Fig. 32.8) (132,133). The combination of HCV and alcohol predisposes to more advanced liver injury than alcohol alone (134,135). Compared to patients with ALD without HCV infection, those with ALD and HCV infection have more severe histologic features, have decreased survival, and develop the disease at a younger age (31,136,137). The progression of fibrosis is most rapid in male patients who continue to abuse alcohol (131,138). Although a daily dose of more than 50 g of alcohol has been shown to be a risk factor for fibrosis in patients with HCV infection, even a moderate consumption of alcohol (<50 g/day) has been shown to result in a dose-dependent increase in liver disease (135,139). Despite these observations, the precise toxic threshold is not known and may be lower and nonuniform in patients at risk. The estimated rate of progression is represented in Figure 32.9 modified and adapted from published data (36,131,139,140,141,142). The prevalence of HCV also increases proportionally as the liver injury becomes more severe, with the relative risk for developing cirrhosis estimated at 8.7 in those patients with ALD and anti-HCV (143,144,145). A French study showed that both mortality and the length of hospital stay was longer in patients with alcohol abuse and hepatitis C (142). In addition, the presence of HCV is reported to be the major risk factor for the development of hepatocellular cancer (HCC) (146). In patients with alcoholic cirrhosis, the 10-year absolute cumulative occurrence risk of HCC has been reported to be as high as 81% in anti-HCV–positive patients with alcoholic cirrhosis as compared to 19% in anti-HCV–negative patients (135,147,148,149). Although one study has questioned this association between progression of HCV and alcohol consumption (150), patients with hepatitis C should be strongly urged to abstain from alcohol, even in moderate quantities, unless further evidence to the contrary becomes available.



▪ **Figure 32.8** A histogram showing the prevalence of various markers of hepatitis C in patients with different forms of alcoholic liver disease. ELISA, enzyme-linked immunosorbent assay; RIBA, recombinant antigen immunoblot assay; PCR, polymerase chain reaction.



▪ **Figure 32.9** Progression of fibrosis with alcoholic liver disease and hepatitis C. ALD, alcoholic liver disease; HCV, hepatitis C virus.

Diagnosis

The diagnosis of ALD is often made in the context of a history of significant alcohol intake, physical signs of liver disease, and supporting laboratory data (18,151,152). However, denial of alcohol abuse is significant (153,154). Physicians usually identify less than 50% of their patients with drinking problems and institute specific recommendations even less frequently (155,156,157). Both the physical findings and laboratory evidence for ALD may be absent or nonspecific, especially in patients with mild ALD or early cirrhosis (19,158,159,160). Therefore, the clinician has to rely on indirect evidence of alcohol abuse, such as questionnaires, information from family members, or nonhepatic laboratory tests to suggest or strengthen a clinical suspicion of ALD (161,162).

General Screening

The historical features that suggest alcohol abuse or alcohol dependence include the amount of alcohol ingested, the social and psychological consequences of alcohol abuse, presence of other alcohol-related diseases, and past incidents of trauma (e.g., frequent falls, lacerations, burns, fractures, or emergency department visits) (155,163). The diagnosis of ALD consists of

documentation of alcohol abuse and evidence of liver disease (19,118). Both clinical and supporting laboratory data for liver disease and alcohol abuse are required for this. Furthermore, alcohol may be one of a number of factors causing the liver disease and the specific contributory role of alcohol alone may be difficult to assess

in a patient with multifactorial liver disease. Biochemical tests have been considered to be less sensitive than verbal tests in screening for alcohol abuse (164,165). Biochemical tests have, however, been considered useful in identifying relapse after abstinence (166,167).

A combination of patient self-reports with corroboration from family or close friends is considered essential in the diagnosis of alcohol abuse. Various questionnaires that have been used in the past for the detection of alcohol dependence and abuse include the CAGE (cut down, annoyed, guilty, and eye-opener) and the MAST (Michigan Alcoholism Screening Test) (164,168). The Alcohol Use Disorders Identification Test (AUDIT) is a ten-item questionnaire that has been discussed in detail in the previous editions of this book (Table 32.5) (169). The major perceived limitation of the full AUDIT questionnaire is the length of time required to administer it. A number of brief screening instruments have been developed, including CRAFFT (Car, Relax, Alone, Forget, Friends, Trouble), brief AUDIT, and RAPS4 (Rapid Alcohol Problems Screen 4) (170,171). The details of these instruments is beyond the scope of this review and have been discussed elsewhere (172). The brief screening questionnaire, RAPS4, has been compared to CAGE in patients from the National Alcohol Survey (173). The RAPS4 had a higher sensitivity (0.86) compared with CAGE (0.67). Both had similar specificity (0.95 vs. 0.98) across gender and ethnicity. Comparing brief versions of the AUDIT to the full AUDIT in the primary care setting showed variable sensitivity and specificity of the brief versions (171). The use of any of these brief instruments cannot be recommended to replace the full AUDIT at the present time. A quantity frequency (QF) score that included questions on drinking frequency was added to RAPS, and the combined instrument, RAPS-QF, outperformed CAGE. It appears that the QF will be a major component of brief questionnaires that are being developed to replace the full AUDIT. Clinical differentiation of the various stages of ALD is difficult at times, and there is no single marker that definitively establishes the etiology of liver disease to be alcohol and laboratory tests are often needed to confirm the diagnosis.

Physical Examination

On physical examination, patients with ALD may show a constellation of abnormalities, which are related to portal hypertension (e.g., ascites, splenomegaly, abdominal wall collaterals, and a venous hum), alcohol abuse and hepatic injury (e.g., cutaneous telangiectasia, palmar erythema, finger clubbing, Dupuytren's contractions, and peripheral neuropathy), and feminization (e.g., gynecomastia and hypogonadism). Detailed discussions of the physical signs have been published recently and are not discussed here (19,165,174).

Although some of the physical findings are more commonly observed in ALD (e.g., parotid enlargement, Dupuytren's contracture, and especially those associated with alcohol abuse and feminization) than in non-ALD, no single physical finding or constellation of findings is 100% specific or sensitive for ALD (175). Furthermore, there is significant interobserver variability for most of these physical findings, which is dependent on the experience of the examiner and the physical finding being sought (174,175). When present, certain findings on physical examination such as ascites, poor nutritional status, and cutaneous telangiectasia indicate significant liver injury and poor prognosis (176,177,178). However, even in the absence of significant liver injury, physical findings associated with alcohol abuse may be present and then subsequently ameliorate with abstinence. Physical examination of the liver, which may be normal in the presence of ALD, does not provide an accurate information about liver volume (179). Its major role is to define the characteristics of the consistency of the liver's lower edge rather than to delineate disease etiology or liver volume.

Other organ dysfunctions in ALD include cardiomyopathy, skeletal muscle wasting, pancreatic dysfunction, and alcoholic neurotoxicity that may coexist with ALD, and evidence of these must be sought during the clinical examination so that appropriate treatment may be provided (180,181,182).

Therefore, the physical examination is unable to either establish the diagnosis of ALD on its own or delineate ALD from non-ALDs and must be considered in the context of the patient's history and laboratory findings.

Laboratory Abnormalities

The various laboratory tests to diagnose ALD are shown in Table 32.6. Low sensitivity and

specificity limit the usefulness of γ-glutamyl transferase (GGT) to diagnose alcohol abuse (183,184,185). Serum transaminases have been used in the diagnosis of ALD. (186)Serum aspartate aminotransferase (AST) is raised only two to six times in severe acute alcoholic hepatitis. Levels of AST more than 500 IU/L or levels of alanine aminotransferase (ALT) more than 200 IU should suggest an etiology other than alcoholic hepatitis. In approximately 70% of patients, the AST/ALT ratio is higher than 2 but this is of greater value in noncirrhotic patients. Ratios greater than 3 are essentially diagnostic for ALD. Macrocytosis is seen in individuals abusing alcohol but lacks sensitivity. The specificity for the diagnosis of ALD is greater with increasing values of the ratio. A combination of raised GGT and mean corpuscular volume (MCV) has a sensitivity of 30% to 40% for diagnosing alcohol abuse. Serum carbohydrate-deficient transferrin (CDT) is a specific and sensitive

test for alcohol use independent of liver disease but is more accurate in men ingesting over 60 g of alcohol daily (187). Frequent determination of serum ethanol levels during the patient's office visits is another simple but often-ignored method of assessing for alcohol use. Other sensitive indices include fucosylated haptoglobin and serum secretory immunoglobulin A (IgA), which need prospective testing at multiple centers before their validity can be confirmed (188,189,190). A direct comparison of four markers of alcohol consumption showed that the overall accuracy of GGT and CDT was highest to detect alcohol drinking while sialic acid and *N*-terminal peptide of type III procollagen (PIIINP) were of value in differentiating liver disease from alcohol abuse (191). Serum hyaluronic acid has been used alone (192) and in combination with other tests (193) to detect the stage of liver disease in ALD. These and other promising biomarkers require additional studies in this area (31).

Table 32.5. Alcohol-Use Disorders Identification Test (AUDIT) Questionnaire Consisting of Ten Questions with Scores Assigned to the Answers

1. How often do you have a drink containing alcohol?
   0—Never
   1—Monthly or less
   2—Two to four times a month
   3—Two to three times a week
   4—Four or more times a week
2. How many drinks containing alcohol do you have on a typical day when you are drinking?
   0—One or two
   1—Three or four
   2—Five or six
   3—Seven to nine
   4—Ten or more
3. How often do you have six or more drinks on one occasion?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly
   4—Daily or almost daily
4. How often during the last year have you found that you were not able to stop drinking once you had started?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly
   4—Daily or almost daily
5. How often during the last year have you failed to do what was normally expected from you because of drinking?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly

4—Daily or almost daily

6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly
   4—Daily or almost daily
7. How often during the last year have you had a feeling of guilt or remorse after drinking?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly
   4—Daily or almost daily
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?
   0—Never
   1—Less than monthly
   2—Monthly
   3—Weekly
   4—Daily or almost daily
9. Have you or someone else been injured as a result of your drinking?
   0—No
   2—Yes, but not in the last year
   4—Yes, during the last year
10. Has a relative, friend, doctor, or other health worker been concerned about your drinking or suggested that you should cut down ?
    0—No
    2—Yes, but not in the last year
    4—Yes, during the last year

Table 32.6. Typical Laboratory Abnormalities in Alcoholic Liver Disease

1. Serum enzymes
   - AST>>ALT
     AST usually <500 IU and ALT <200 IU
   - Alkaline phosphatase and γ-glutamyl transpeptidase
     Levels of both usually elevated to a variable degree
2. Igs
   - Levels of both IgG and IgA are elevated
3. Metabolic alterations
   - Hyperglycemia
   - Hypertriglyceridemia
   - Hyperuricemia
   - Electrolyte abnormalities
     Low levels of potassium, magnesium and phosphorus
4. Tests of liver function
   - Serum albumin, prothrombin time, and serum bilirubin usually normal until significant liver injury is present
5. Hematologic abnormalities
   - Mild anemia common (usually macrocytic)
   - Platelets (normal to markedly decreased)
   - Elevated white blood cell count
     Leukemoid reactions associated with alcoholic hepatitis

AST, aspartate aminotransferase; ALT, alanine aminotransferase; Ig, immunoglobulin.

In symptomatic patients, nonspecific findings include elevated levels of uric acid, lactate, and triglycerides and a decrease in the concentration of magnesium, glucose, phosphate, and potassium. Polyclonal hyperglobulinemia and increase in circulating IgA level also occurs with ALD (194). It must be reiterated that no specific laboratory test exists that is specific for ALD.

Nonalcoholic steatohepatitis (NASH) is a distinct clinical entity with histologic features suggestive of alcohol abuse with little or no alcohol ingestion (195). This is seen in association with other features of the metabolic syndrome (196). There are many similarities in the pathogenesis of NASH and alcoholic steatohepatitis, but the critical component of the injury in ALD is consumption of significant amounts of alcohol. There are no laboratory tests or histologic patterns that reliably differentiate NASH from alcoholic steatohepatitis.

Liver Biopsy in Alcoholic Liver Disease

A liver biopsy is useful but not essential in the management of ALD (197). The role of liver biopsy in ALD is shown in Table 32.7. A liver biopsy is helpful in establishing the diagnosis because as many as 20% of patients with alcohol abuse have non-ALD or a coexisting etiology for liver disease (198). Clinical and biochemical indicators are poor markers of the severity of liver disease, and a biopsy is needed to establish the stage and severity of liver disease (197,199). A biopsy assumes greater importance in patients who continue to have abnormal liver test results after a period of abstinence of approximately 3 to 4 months. Liver biopsy is usually safe, with a low morbidity and mortality, but precautions taken should be the same as that for any other patient undergoing a liver biopsy. In patients with a diagnosis of acute alcoholic hepatitis with a coagulation profile precluding a percutaneous liver biopsy, consideration should be given to the transjugular biopsy.

The histologic features of alcohol-induced hepatic injury include steatosis (fatty change), lobular hepatitis, periportal fibrosis, Mallory bodies, nuclear vacuolation, bile ductal proliferation, and fibrosis or cirrhosis (17). Development of large-droplet (macrovesicular) steatosis (fatty liver) is the earliest and most common manifestation of ALD. It is often clinically asymptomatic and completely reversible on abstinence (200). It is most prominent in the centrilobular regions but in severe cases may involve the entire lobule (201). It

is most commonly diagnosed on ultrasound, demonstrating fatty change with increased echogenicity of the liver parenchyma (202).

Table 32.7. Role of Liver Biopsy in Alcoholic Liver Disease

Diagnosis

- Confirm diagnosis
- Exclude other causes of liver disease (primary or concomitant)
- Assess extent of liver damage

Prognosis (adverse)

- Neutrophilic infiltration
- Stenosis of central veins

- Number of hepatic stellate cells, giant stellate cells, and lipid vesicles in stellate cells
- Number of Kupffer cells
- Degree of liver cell necrosis
- Perivenous fibrosis

Therapeutic decision

- Before corticosteroid therapy

Alcoholic hepatitis is characterized by liver cell damage, neutrophilic infiltration of the lobules, and fibrosis (22,178). Steatosis and hepatitis may exist independent of each other and do not imply a continuum of changes. The liver cell damage in alcoholic hepatitis includes hepatocyte necrosis, ballooning degeneration, and lobular inflammation that affect the perivenular regions in the earliest stages (34). Ballooning is secondary to the accumulation of protein and water. Mallory bodies are irregular refractile eosinophilic cytoplasmic structures with a beaded structure and stain positive for ubiquitin (203). They have been shown to be aggregated cytokeratin intermediate filaments and other proteins. Mallory bodies, giant mitochondria, neutrophilic infiltration, and fibrosis may also be seen in conditions other than ALD (204). In contrast to their presence in simple fatty liver, megamitochondria in alcoholic hepatitis have been reported to be associated with a milder form of alcoholic hepatitis, a lower incidence of cirrhosis, and fewer complications with a good long-term survival (205). Alcoholic hepatitis is associated with perivenular and pericellular fibrosis as a consequence of stellate cell activation. Compared to patients who consume alcohol and have normal hepatic histology, inactive cirrhosis, or active alcoholic cirrhosis, those with acute alcoholic hepatitis without cirrhosis have a 2- to 5-fold increase in the number of hepatic stellate cells (HSCs) and a 7- to 15-fold increase in number of giant HSCs. There is also a marked increase (15- to 20-fold) in the number of lipid vesicles in giant HSCs (206). These changes are associated with an increase in the extent of perisinusoidal collagenization. Perivenular fibrosis is considered to be a harbinger of future cirrhosis, especially in patients who continue to abuse alcohol or those who are coinfected with HCV (26,145). Sclerosing hyaline necrosis is a more extensive degree of alcoholic hepatitis and is associated with extensive fibrosis (207). The number of Kupffer cells and serum levels of TNF-α, IL-6, and IL-12 were significantly higher in patients with ALD than in controls (208). In patients with severe steatohepatitis, stenosis of the central veins and the degree of stenosis correlate with the amount of ascites, hyperbilirubinemia, and peripheral leukocyte counts (209). Histologically, the Mallory bodies appear to increase in size significantly as veno-occlusive lesions become more severe, but these lesions do not correlate with the degree of sinusoidal neutrophilic infiltration.

There are a number of nonalcoholic hepatic disorders and conditions (e.g., NASH, total parenteral nutrition, and certain drugs—corticosteroids, amiodarone, and synthetic estrogens) that may mimic ALD histologically and these have been reviewed extensively elsewhere (210,211). In particular, it may be necessary to perform a liver biopsy to identify other diseases that may mimic ALD clinically. Histologic differentiation between these diseases may be difficult. Perivenular fibrosis, which is similar but not identical to that often described in association with ALD, has been described in hepatitis C infection (143,212,213). This emphasizes the need for a detailed evaluation of the histology of and appropriate clinical correlation in these diseases. Excessive iron accumulation with siderosis of both the hepatocytes and the Kupffer cells occur in up to two thirds of patients with ALD (214). This is secondary to increased intestinal absorption, prior transfusion, or rarely spur cell anemia (215). A liver biopsy should be performed in patients who have biochemical findings suggestive of iron overload (total iron-binding capacity [TIBC] saturation >45%) to rule out hereditary hemochromatosis by calculating the hepatic iron index because both HCV infection and ALD can each individually cause biochemical abnormalities suggestive of hereditary hemochromatosis (216,217). In the alcoholic patient without cirrhosis, the presence of moderate periportal hemosiderosis should warrant testing for HFE (the abnormal gene in most patients with hemochromatosis) in addition to biochemical tests of iron overload (i.e., serum iron, TIBC, and ferritin)(217).

Imaging Studies in Alcoholic Liver Disease

Imaging studies have been used to diagnose the presence of liver disease but may not have a specific role in establishing alcohol as the etiology of liver disease. The diagnosis of fatty change, established cirrhosis, and hepatocellular carcinoma is suggested by ultrasound or computed tomography (CT) scan and confirmed by other laboratory investigations (197,218). Altered echogenicity of the liver has been considered to be moderately sensitive but not specific for the diagnosis of the fatty change associated with alcoholic liver injury. Established cirrhosis can be suspected on CT scan or magnetic resonance imaging on the basis of the findings of a lobular surface of the liver with altered density of the liver, but the etiology cannot be established with certainty. Enlargement of the caudate lobe and right posterior notch has been suggested to identify alcohol as the etiology of liver disease (219). Evidence of portal collaterals and dilated portal–splenic axis suggests the presence of associated portal hypertension (220). The major value of imaging studies is to exclude other causes of abnormal liver function test results in a patient who abuses alcohol such as obstructive biliary pathology, and infiltrative and neoplastic diseases of the liver (221). Another tool for the diagnosis of ALD is hepatic phosphorus 31 magnetic resonance spectroscopy (222). This is used to calculate hepatic energy metabolism and phospholipid membrane metabolism.

Lower phosphodiesterase to adenosine triphosphate (ATP) ratios have been reported in patients with alcoholic cirrhosis.

Endoscopy in Alcoholic Liver Disease

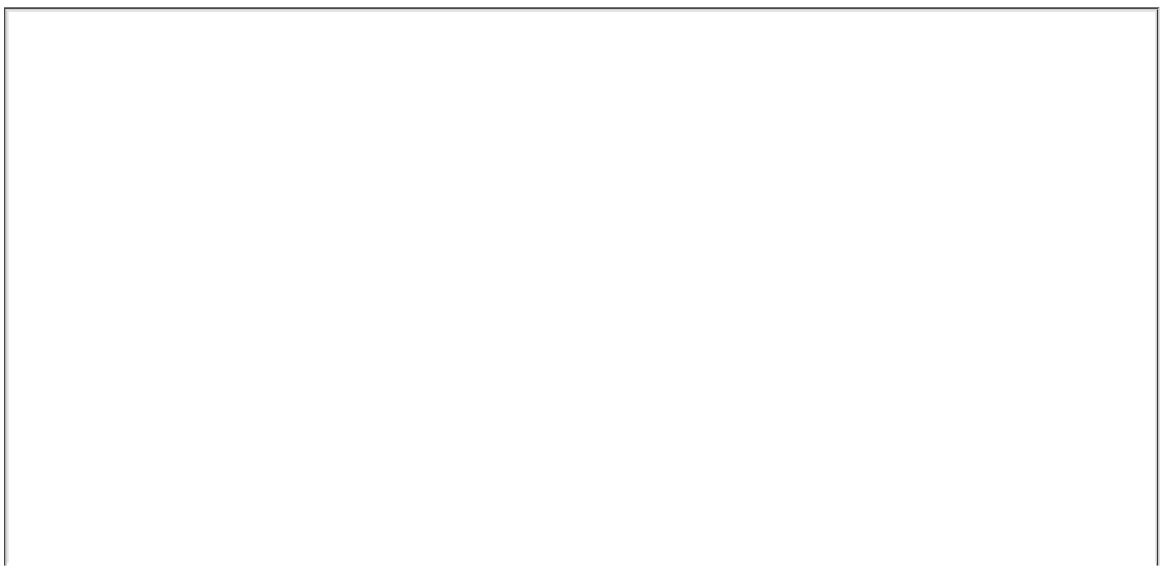
An upper gastrointestinal endoscopy in patients with ALD is used electively to establish the presence of esophagogastric varices and emergently to identify the source of gastrointestinal bleeding. In the clinical situation of alcohol abuse, upper gastrointestinal bleeding may be secondary to a variety of causes that include Mallory-Weiss tear, peptic ulcer disease, direct alcohol-induced gastric mucosal erosions and injury, portal hypertensive bleeding from varices, or congestive gastropathy secondary to cirrhosis (223,224). Endoscopy has both diagnostic and therapeutic value in an alcoholic patient with active upper gastrointestinal bleeding.

Pathogenesis

An in-depth discussion of the pathophysiology of alcohol-induced liver disease is beyond the scope of this chapter and recent major reviews are recommended (18,47,201,225). However, a general understanding of the mechanisms of alcohol-mediated liver damage is essential because it forms the basis of current therapeutic strategies.

In the past, it has been considered that whole liver tissue analysis would provide an understanding of the pathogenesis of ALD. However, the liver consists of both hepatic parenchymal cells (65%) and nonparenchymal cells (e.g., endothelial cells, Kupffer cells/hepatic macrophages, HSC, bile duct epithelial cells, and pit cells/liver natural killer cells)(226,227,228,229). All these cellular components are involved in the pathogenesis of ALD (Table 32.8). Although the nonparenchymal cells constitute less than a third of the liver volume, they have distinct functions and are

involved in cellular homeostasis, hepatocyte support, chemokine release and hepatocyte injury, fibrosis, and cell death (206,208,227,230,231).



Target	Mediator	Consequence
HEPATOCYTES		
Cytochrome P450IIE1	Reactive oxygen species	Lipid peroxidation
Abnormal methionine metabolism		
SAMe, *S*-Adenosyl Homocysteine	Transmethylation reaction	Nucleic acid, protein, phospholipids
Mitochondria	GSH depletion	Reactive oxygen species
	Membrane fluid abnormality	Megamitochondria
	Electron transport chain	Defective ATP depletion
	Mitochondrial DNA breaks	Apoptosis induction
Hepatocyte growth	Impaired protein synthesis, Inhibits calcium mobilization, Decreased DNA synthesis	Impaired regeneration
Increased expression of proto-oncogenes	Increased regenerative proliferation	Hepatocellular carcinoma
Matrix remodeling and deposition	Hepatocyte communication	Impaired apoptosis, regeneration
KUPFFER CELL/MACROPHAGES		
Alcohol-induced damage	Cytokine release	Increased TNF-α expression
	Defective IL-10 release	Increased apoptosis
	Defective regulation of local inflammation	Hepatocyte inflammatory cell infiltration
Iron overload (increased absorption)	Impaired function	Endotoxemia
SINUSOIDAL ENDOTHELIAL CELLS		
Inflammatory cell, Migration	ICAM expression increased	Hepatocyte injury
Hypoxemia	Microvascular circulation abnormalities	
HEPATIC T LYMPHOCYTES		
Increased activation	TNF-α release	Hepatocyte apoptosis
HEPATIC STELLATE CELLS		
Oxidative stress, Endotoxemia, Differential MAT expression	Activation	Lobular fibrosis

SAMe, *S*-adenosyl methionine; GSH, glutathione; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; TNF, tumor necrosis factor; IL, interleukin; ICAM, intercellular adhesion molecule; MAT, methionine adenosyl transferase.

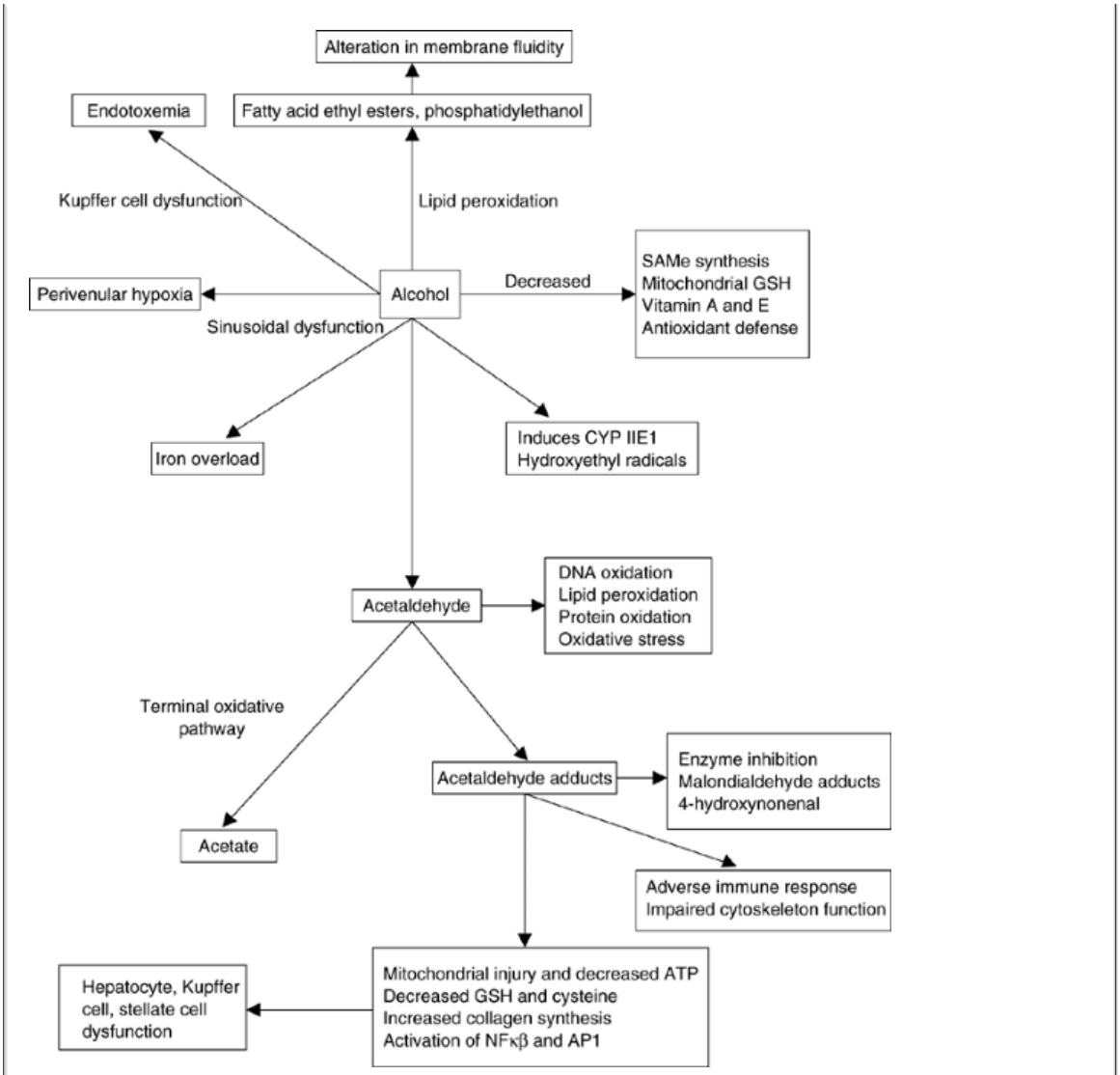
Table 32.8. Role of Cellular and Subcellular Organelles in the Pathophysiology of Alcoholic Liver Disease

Alcohol-induced liver injury is the result of a complex interaction between alcohol metabolism, and inflammatory and immune responses that result in cellular injury. These include oxidative stress and the consequent inflammatory cellular response that cause direct injury to hepatocytes in addition to activation of hepatic nonparenchymatous cells (Fig. 32.10). Additionally, indirect damage to the liver occurs because of endotoxin/cytokine activation, immune-mediated mechanisms, and induction of fibrogenesis (232). Superimposed on these hepatotoxic mechanisms is the impairment of hepatic regeneration with alcohol use, although studies on rats have questioned this (233,234,235).

Direct Hepatocyte Injury

Hepatocytes are the primary site of alcohol oxidation, and there are two major oxidative pathways

involved: The alcohol and aldehyde dehydrogenase pathway and the microsomal ethanol-oxidizing system (MEOS), the major component of which is CYP 2E1 (Fig. 32.10) (225,236). Therefore, ethanol oxidation generates reactive free radicals, leading to oxidative stress that is reinforced by the depletion of glutathione (GSH) and vitamin E (antioxidants) (237,238). Oxidative stress induces the transcription of several cytokines and release of growth factors from the different cells in the liver that interact with the immune cells mediating hepatocyte injury (228,232).



▪ **Figure 32.10** Schematic of the role of the hepatocyte and alcohol oxidative pathways in the pathogenesis of alcoholic liver disease. SAMe, *S*-adenosyl methionine; GSH, glutathione; DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; NF, nuclear factor; AP, activator protein.

Alcohol dehydrogenase pathway

ADH is an enzyme that produces acetaldehyde, which causes direct hepatocyte damage and immune-mediated damage by the formation of acetaldehyde adducts that are potent immunogens (121,239). In addition to acetaldehyde, other aldehydes produced as a result of free radical generation include malondialdehyde (MDA) that has been shown to combine with the cytochrome *c* oxidase subunits IV and V of mitochondria (121). Anti–acetaldehyde adduct IgA and IgG, as well as anti-MDA adduct antibodies, were found in a significantly higher number of patients with severe alcoholic hepatitis and cirrhosis (194). Additionally, acetaldehyde and MDA can form hybrid adducts with proteins (mercaptoacetic acid adducts) that are also highly immunogenic (240). These suggest that the ADH pathway provides a strong immunologic mechanism in the pathogenesis of ALD. Acetaldehyde also significantly enhances the DNA binding of two major transcription factors: Nuclear factor κβ (NFκβ) and activator protein-1 (AP-1) and increases their transactivating activities (241). These observations suggest that acetaldehyde plays a major role in regulating the expression of proinflammatory cytokines by activating NFκβ and AP-1 (242).

Cytochrome P4502E1 pathway

Cytochrome P4502E1 (CYP 2E1) is a component of the MEOS enzyme pathway that plays a major role in inducing oxidative stress and alcohol-induced hepatocellular damage (243). After chronic

ethanol ingestion, a 5- to 10-fold induction of CYP 2E1 occurs that not only metabolizes ethanol but is also responsible for the activation of xenobiotics to toxic metabolites (104,106,243). This increases the vulnerability of patients who abuse alcohol to solvents, xenobiotic compounds used in industry, and therapy, as well as vitamin A precursors (i.e., β-carotene and retinol) (244,245,246). In contrast to the ADH pathway that is not inducible in humans, CYP 2E1 is induced in humans by ethanol ingestion; it increases the production of ROS and the consequent membrane peroxidation that results in cell damage (236,247). There is increased generation of superoxide radical and free radical production including the 1-hydroxyethyl free radical intermediates (129,248). Normally, cellular protective mechanisms against oxidative stress prevent the adverse effects of these ROS (249). In the presence of liver disease and malnutrition, with the maximal activation of the system, the detrimental responses predominate and result in tissue injury (250). Other cytochromes such as the P-450 1A2 and 3A4 are also induced and may contribute to the cell injury and ROS generation associated with ethanol ingestion (104).

The CYP 2E1 system may not be as essential as other cytochrome enzymes in the pathogenesis of ALD (251). CYP 2E1 knockout mice have been shown to be as susceptible to the hepatoinjurious effects of ethanol (252). Induction of other cytochrome enzymes (i.e., CYP 1A, CYP 2A, CYP 2B, and CYP 3A) also occur with ethanol and may contribute to the oxidative damage associated with ethanol ingestion. Dissociation of CYP 2E1 and ALD is also suggested by the prevention of experimental ALD with gadolinium chloride despite the induction of CYP 2E1 (253). Nonetheless, most data suggest that CYP 2E1 is involved in the pathogenesis of ALD (226). Furthermore, studies that have attempted to question the role of CYP 2E1 have examined the early stages of ALD, whereas CYP 2E1 plays a role later in the progression of ALD. Finally, CYP 2E1 is much lower in mice than rats or humans and caution must be exercised before extrapolating the findings in mice to humans (226).

Abnormal methionine metabolism

The liver plays a central role in methionine metabolism with the formation of *S*-adenosyl methionine (SAMe) in the presence of the enzyme methionine adenosyl transferase (MAT) (254). An inactivation of hepatic MAT occurs in ALD with the depletion of hepatic SAMe and GSH and decreased transmethylation reactions. These result in impaired antioxidant defense, altered phospholipid composition and membrane fluidity, and possibly altered DNA stability (47). Finally, the homocysteine released as a consequence of altered MAT activity may stimulate HSCs and promote hepatic fibrosis (255,256).

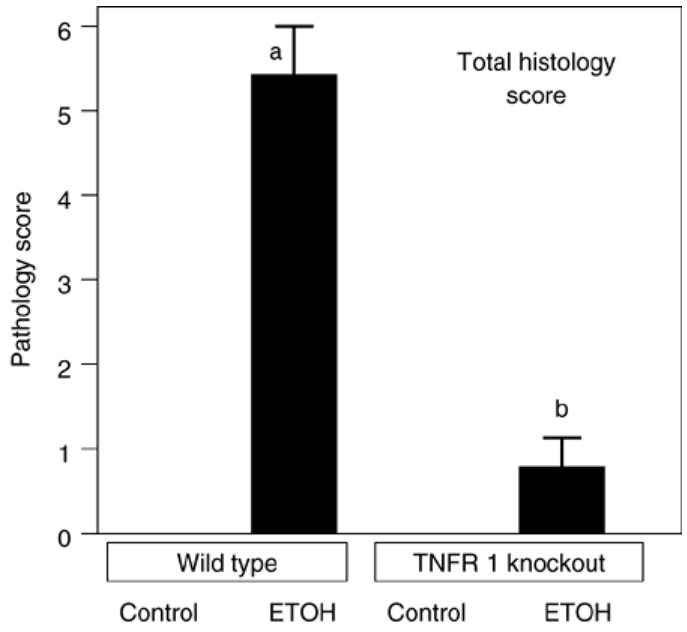
Role of hepatic mitochondria

Mitochondrial dysfunction has been observed in the alcohol-fed animal models (257). Increased synthesis of ROS, decreases in mitochondrial membrane potentials, increased oxidative modification, and single-strand breaks in mitochondrial DNA have been reported (257,258). These may be inducers of apoptosis and result in hepatocyte death in ALD. Dysregulated apoptosis has been suggested as a key pathogenic

event in ALD, with activation of effector caspaces (228,259,260,261).

Indirect Hepatocyte Injury

Hepatic macrophages (Kupffer cells) generate cytokines (such as TNF-α and transforming growth factor-β) that may accentuate or promote liver cell injury (27,228,262). The role of TNF-α in alcoholic hepatitis is not clear (263). Elevated serum levels of TNF-α in alcoholic hepatitis has been correlated with disease severity and mortality (122). TNF-α receptor 1 knockout mice are resistant to alcohol-induced liver damage, as shown in Figure 32.11 (264). These studies suggest a role for TNF-α in the pathogenesis of liver injury in ALD. The contribution of Kupffer cells is supported by an attenuated alcohol-mediated hepatic injury by blocking Kupffer cell function (265). Furthermore, MAT is expressed in both Kupffer cells and sinusoidal endothelial cells, and an alteration in the expression of this enzyme system in the three groups of cells (i.e., hepatocytes, macrophages, and endothelial cells) could contribute to the pathogenesis of injury in ALD (226). Liver-associated T lymphocytes mediate injury and these may be responsible for both immune-mediated and non–immune mediated (through release of cytokines) hepatic injury (266). HSCs may be responsible for both matrix remodeling and regulation of local inflammation (206). Alteration in stellate cell activation may result in fibrosis and cell death in ALD (267,268). Products of lipid peroxidation have the ability to activate the HSC and stimulate fibrogenesis (269). The contribution of nonhepatic cellular components to hepatic injury is not entirely understood and warrants further studies to examine the role of these cell types.



▪ **Figure 32.11** TNF receptor 1 (TNFR 1) knockout mice are less prone to alcohol-mediated hepatic injury compared to wild-type control mice. ETOH, ethanol. *a*, *P* <.001 versus control; *b*, *P* <.01 versus control.

Prognostic Factors

Alcoholic cirrhosis has a worse prognosis than other forms of cirrhosis, as shown in Table 32.9 (270). A number of adverse prognostic factors for the long-term outcome in ALD have been suggested, and these include the development of cirrhosis, ascites, portal hypertension (especially with evidence of encephalopathy), spontaneous bacterial peritonitis, hepatic encephalopathy, hepatorenal syndrome, coagulopathy, severe hyperbilirubinemia, hypoalbuminemia, severe malnutrition, decreased galactose elimination capacity, evaluation of α-fetoprotein, increasing age, histological evidence of hepatic inflammation, continued alcohol abuse, and coinfection with other hepatotropic virus infection (18,271,272,273,274). Iron overload and bacterial infections also predict poor outcome in ALD (275,276).

Scoring System for Disease Severity

Clinical progress in the therapy for ALD has been limited in part by the lack of accurate predictors of prognosis. This has also resulted in difficulty in comparing outcomes and results of different treatments because of the nonhomogeneity in the predictors used. Accurate prediction of the prognosis of the most severe form of ALD, alcoholic hepatitis, is necessary to develop reliable treatment strategies for this disease. The modified Maddrey discriminant function (DF) that includes prothrombin time and bilirubin levels is the most widely used predictor of severity of alcoholic hepatitis (277). Values of 32 and more are associated with a mortality

of 35% to 40% and are used to identify patients who will benefit from corticosteroid therapy. Patients with a DF less than 32 had a survival of 83% to 90% and they do not benefit from corticosteroids (278). Presence of encephalopathy has been included by some workers to predict outcome and the decision to start corticosteroids (279). The Model for End-Stage Liver Disease (MELD) and the Glasgow alcoholic hepatitis score (GAHS) have been used more recently to predict the outcome in patients with acute alcoholic hepatitis (279,280,281). A MELD score of 21 or higher had the best sensitivity and specificity in predicting 90-day mortality (282). Other authors have also used MELD, and a score of 11 has also been used as a cutoff to predict the outcome (283). These studies have compared MELD with Maddrey DF and either found them to be equally sensitive and specific or found MELD to be superior to Maddrey DF in predicting the outcome in acute alcoholic hepatitis (279). The limitations of MELD include the difficulty in its calculation at the bedside, the variation of the predictive value among studies, and the inclusion of serum creatinine that limits its usefulness in the presence of hyperbilirubinemia, which reduces the accuracy of

creatinine measurement (284). The GAHS has been validated in a large population of patients studied up to day 84, allowing the assessment of not only the short-term outcome on days 28 to 30 but also the clinical course of the disease (281). The key components of GAHS include age, white blood cell count, blood urea, prothrombin time, and serum bilirubin (280,281). It is still unclear whether GAHS will be universally applied, and both MELD and GAHS need to be extensively studied by different groups before they are accepted as predictors of outcome and replace the widely used Maddrey DF.

Table 32.9. Survival of Different Types of Cirrhosis

Etiology	*N*	5 year (%)	10 year (%)
Alcohol	82	23[a]	7[a]
Cryptogenic	13	33	20
HCV	62	38	24
HBV	42	48	20
Hemochromatosis	20	41	22
Autoimmune	16	46	23
PBC	36	56	39

[a]*P* <0.05 versus other forms of cirrhosis.
HCV, hepatitis C virus; HBV, hepatitis B virus; PBC, primary biliary cirrhosis.
Adapted from Pessione F, Ramond MJ, Peters L, et al. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. *Liver Int* 2003;23(1):45–53.

Abstinence

Abstinence is a major goal of therapy and remains a fundamental factor in the management of ALD (270,285). It is perhaps the most important but difficult therapeutic intervention for ALD (225,286). Abstinence has been shown to improve the outcome and histologic features of hepatic injury, reduce portal pressure, and decrease progression to cirrhosis (Table 32.10), but this may be lower in female patients (58,209,272,287,288,289,290,291). This improvement can be relatively acute, and in 66% of patients abstaining from alcohol, significant improvement was observed in 3 months (292). Continued alcohol ingestion results in increased portal hypertensive bleeding, especially in patients who have bled in the past, and worsens both short- and long-term survival (224). The deleterious effect of continued alcohol ingestion on the outcome compared to the abstinent population is shown in Figure 32.12. Furthermore, a survival analysis of abstinent alcoholic patients with cirrhosis who are infected with HCV compared to those with HCV cirrhosis who had never consumed alcohol showed no difference (293).

Table 32.10. Five-Year Survival in Alcoholic Liver Disease (the Effect of Abstinence)

			Survival (%)	
Authors	Time (y)	Number	Stopped drinking	Continued drinking

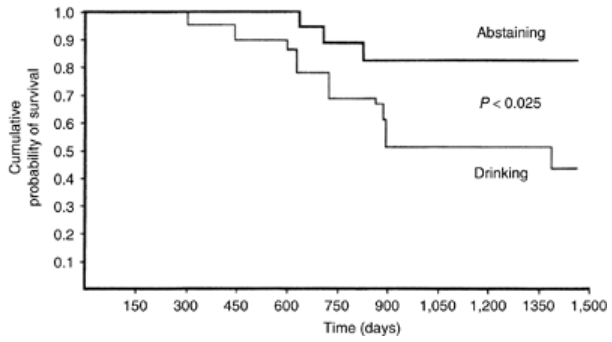
Pessione et al. (270)	5	122	57[a]	27
Merkel et al. (272)	4	45	87	55
Borowsky et al.[b] (287)	3	54	45[a]	20
Brunt et al. (288)	7	258	77[a]	48
Juhl and Tygstrup (290)	8	168	70[a]	44
Powell and Klatskin (291)	12	278	63[a]	40

Figures in parentheses are the reference number in bibliography.
[a]$P < 0.05$ versus continued drinking, as stated in the text or estimated from Life Table Analysis.
[b]Data in Borowsky's study was for 2-year survival for heavy drinking versus total abstinence.

Recidivism is the major risk in all patients at any time after abstinence (294,295). No controlled trials exist on the benefits of alcohol abstinence and the effects of recurrent drinking on ALD. Cohort studies, however, have consistently demonstrated that abstinence does improve survival at all stages of the disease (270,287). Several drugs have been tried to help sustain abstinence. Naltrexone, a pure opioid antagonist controls the craving for alcohol. A Cochrane systematic review of the use of naltrexone and nalmefene in 29 randomized controlled trials concluded that short-term treatment with naltrexone lowers the risk of relapse of alcohol abuse (296). Acamprosate (acetylhomotaurine) is a novel drug with structural similarities to excitatory amino acids and γ-aminobutyric acid and is associated with reduction in withdrawal symptoms (297). In 15 controlled trials, the efficacy of acamprosate has been demonstrated in reducing withdrawal symptoms but the effects on survival are not yet available (298).

Exercise has been shown to ameliorate the alcohol-induced hepatic injury in rats (299). It is possible that a healthy behavior pattern in humans would reinforce abstinence in addition to its beneficial effect on ALD (52). Human studies should be encouraged given the multiple beneficial effects of exercise. Caution must, however, be exercised before recommending exercise

in patients with ALD because of the possible underlying cardiomyopathy in these patients.



▪ **Figure 32.12** Effect of abstinence on long-term survival (Kaplan-Meier survival curve).

Hepatic Inflammation

The presence of hepatic inflammation appears to be the single most important prognostic histologic factor in patients with ALD (18,300,301). In a study of 217 patients (140 with cirrhosis and 77 without cirrhosis) with biopsy-proved ALD, the presence of hepatitis indicated a poor prognosis (300). Patients with cirrhosis and hepatitis had increased the 1- and 5-year mortality rates to 27% and 47%. This contrasts with the survival rates in patients with cirrhosis without hepatitis are similar to those with no cirrhosis or hepatitis. These data have been indirectly confirmed in cases in which the presence of polymorphonuclear cells on liver biopsy was found to be a prognostic factor for early and late survival (273).

Hepatitis C

As discussed previously, the presence of HCV is another factor that causes inflammation in these patients (302,303). The decision to use antiviral therapy in patients with ALD and HCV infection should be determined on an individual basis. Because the data available indicate that active drinking will reduce the efficacy of interferon therapy for HCV infection, the potential use of interferon or other antiviral agents must be decided on an individual basis (304,305,306). However, the potential efficacy of antiviral therapy may be improved by abstinence, and therefore, the potential for HCV therapy may be a motivational factor (305).

Interaction with other drugs

The activation of the microsomal oxidative enzyme pathways also enhances the hepatotoxicity of other drugs, especially acetaminophen. A combination of enhanced cytochrome P-450 system and decreased hepatic GSH results in severe toxicity. A significant proportion (64%) of patients who developed acetaminophen toxicity have been reported to be alcohol abusers (307,308). Furthermore, most patients reported taking doses that were well below the accepted toxic range (309). Awareness and suspicion of the diagnosis are the most important factors in the management of these patients. Diagnostic clues include very high aspartate transaminase levels (>1,000 IU/L) and a prolonged prothrombin time. Early and accurate diagnosis is essential because of the high mortality and need for early therapy (310).

Therefore, continued alcohol abuse, evidence of hepatic inflammation with hyperbilirubinemia, elevated transaminase levels, prolonged prothrombin time, and hypoalbuminemia seem to correlate with the worst prognosis.

Therapy

Therapy is based on the stage of the disease at which therapy is started and the pathogenetic event being targeted (Table 32.11) (225,311). The primary treatment modalities that have been evaluated in the acute phase of alcoholic hepatitis include agents that suppress inflammation (e.g., corticosteroids) and anticytokine therapy (e.g., pentoxifylline, infliximab, and etanercept), nutritional improvement (e.g., enteral and parenteral supplements including amino acids and anabolic steroids), modifiers of metabolism (e.g.,

propylthiouracil [PTU]), and inhibitors of hepatic fibrosis (e.g., colchicine) (47). Once cirrhosis is established, complications of cirrhosis that include evidence of chronic hepatic failure (e.g., encephalopathy, ascites), as well as those of portal hypertension (e.g., variceal bleeding), are treated along similar lines as those in patients with non-ALD, with additional attention given to other organ dysfunctions associated specifically with alcohol (311,312).

Direct injury		Indirect injury	
Proposed treatment	**Mechanism**	**Mechanism**	**Proposed treatment**
PUL	Membrane damage	Intestinal function	Antibiotics, nutrition
		Cytokines	Anti-TNF-α Pentoxifylline[a], steroids γ-interferon
S-adenosyl methionine PTU[b]	Oxidative stress	Immunologic mechanism	Steroids[a] Colchicine[b], PUL[c]
	Hypermetabolism	Fibrogenesis	
	Regenerative capacity Nutrition[c], oxandrolone[c], hepatotropic agents		

Table 32.11. Pathophysiology and Potential Therapies

Treatment of Alcoholic Hepatitis

Therapy for acute alcoholic hepatitis is determined partly on factors that estimate mortality. These indices are calculated on the basis of laboratory tests, and the most commonly used index is the Maddrey DF ([prothrombin time prolongation over controls in seconds × 4.6] + serum bilirubin in mg/dL) (278). A number of previously used predictors including the Child-Turcotte-Pugh score and the combined clinical and laboratory index have fallen out of favor because of low diagnostic accuracy (300,313). Recently, the MELD and GAHS have been gaining interest as accurate predictors of outcome (281).

The accuracy of five extracellular matrix serum markers (e.g., laminin, PIIINP, and type I, type III, and type IV collagens) has been assessed to diagnose the severity of alcoholic hepatitis (314). On the basis of a histologic scoring system, serum laminin and type IV collagen levels were the most accurate in identifying severe alcoholic hepatitis. The sensitivity of serum laminin was 90% and its specificity was 77%, while those of serum collagen type IV were 80% and 77%, respectively. The major role for these markers may be to identify patients with severe alcoholic hepatitis who are being considered for corticosteroid therapy so that a liver biopsy can be avoided. Studies are needed to combine one of the predictors with other tests such as serum matrix markers that may improve the diagnostic accuracy of the severity of alcoholic hepatitis.

Corticosteroids

Rationale

Corticosteroids have been used to suppress inflammatory and immune response directed against the neoantigens induced by acetaldehyde adducts that include liver-specific lipoprotein, liver membrane antigen, Mallory bodies, and epitopes of protein–aldehyde adducts in the liver (315). Specific immune targets that have been considered targets of corticosteroids include MDA and acetaldehyde adducts, autoantibodies to P4502E1 and P4503A4, and antibodies to the liver membrane antigen (316). There is enhanced cytotoxicity of lymphocytes toward hepatocytes in patients with alcoholic hepatitis (228,317) Steroids also exert a direct antifibrotic effect by suppressing the expression of extracellular matrix proteins in the liver (113). Finally, inhibition of cytokine synthesis by glucocorticoids in response to gut-derived endotoxins also contributes to their beneficial effects in ALD.

Clinical trials

Corticosteroids have been the most extensively used mode of treatment of alcoholic hepatitis, but their efficacy still remains controversial. Of a total of 13 randomized controlled trials, 5 showed that corticosteroids

reduce mortality compared with placebo, while 8 others found no difference in outcomes (Table 32.12) (318,319,319,320,321,322,323,324,325,326,327,328,329,330). Although the results are not consistent, differences in study design may explain the different outcomes. These include variability in dose and duration of therapy, selection of patients (e.g., varying time intervals before randomization or inconsistent use of disease severity scoring), possible misclassification (a consequence of inclusion without liver biopsy), and severity of illness, concomitant medical problems, or medications, as well as undiagnosed chronic viral hepatitis infections. Despite these variations, three separate meta-analyses (331,332,333) have found a benefit in the use of steroids and one meta-aggregate (330) suggested no improvement after attempting to control for potential confounders. The results of the combined data from these meta-analyses suggested that corticosteroids should be targeted to the subset of patients with severe disease. In one meta-analysis, steroid treatment provided a protective efficacy in 27% of patients with hepatic encephalopathy, which increased to 40% among higher-quality trials and in 51% of patients without gastrointestinal bleeding (Table 32.13) (332). In patients without hepatic encephalopathy, corticosteroids had no protective efficacy.

Table 32.12. Results of Randomized Controlled Trials of Corticosteroids in Alcoholic Hepatitis

Author	Date	Number of patients	Deaths—placebo (with 95% CI)	Deaths—steroid (with 95% CI)	Relative risk
Helman et al.	1971	37	6/17 (0.35) (0.14–0.62)	1/20 (0.05) (0.0013–0.25)	0.143
Porter	1971	20	7/9 (0.77) (0.44–0.93)	6/11 (0.55) (0.28–0.79)	1
Campra	1973	45	9/25 (0.36) (0.2–0.56)	7/29 (0.35) (0.18–0.57)	1
Blitzer et al.	1977	33	5/16 (0.31) (0.14–0.56)	6/12 (0.5) (0.25–0.75)	1
Lesesne	1973	14	7/7 (1.0) (0.63–1.0)	2/7 (0.29) (0.09–0.65)	0.29
Maddrey et al.	1978	27	6/31 (0.194) (0.09–0.36)	1/24 (0.042) (0.009–0.20)	0.22
Shumaker et al.	1978	27	7/15 (0.47) (0.25–0.75)	6/12 (0.5) (0.25–0.75)	1
Depew et al.	1980	28	7/13 (0.54) (0.29–0.77)	8/15 (0.53) (0.3–0.75)	1
Theodossi et al.	1982	55	16/28 (0.57) (0.39–0.74)	17/27 (0.63) (0.44–0.79)	1
Mendenhall et al.	1984	178	50/88 (0.57) (0.46–0.67)	55/90 (0.61) (0.51–0.71)	1
Bories et al.	1987	45	2/21 (0.095) (0.029–0.29)	1/24 (0.042) (0.0098–0.20)	1
Carithers et al.	1989	66	11/31 (0.36) (0.21–0.53)	2/35 (0.057) (0.108–0.19)	0.16
Ramond et al.	1992	61	16/29 (0.55) (0.37–0.72)	4/32 (0.125) (0.05–0.28)	0.23

Summarized from O'Shea RS, McCullough AJ. Treatment of alcoholic hepatitis. *Clin Liver Dis* 2005;9(1):103–134.

The major limitation of these meta-analyses was that they were not designed to analyze the individual patient data. A reanalysis of the pooled raw data from the three recent placebo-controlled randomized trials, restricted to patients with a DF of 32 or more, concluded that patients treated with corticosteroids had a significantly higher survival at 28 days than those given

placebo—84.6% versus 65% (Fig. 32.13) (277,278). Therapy with corticosteroids, age, and serum

creatinine were the independent predictors of outcome of these patients. On the basis of these data, the number needed to treat was calculated to be five (i.e., five patients treated to prevent one death).

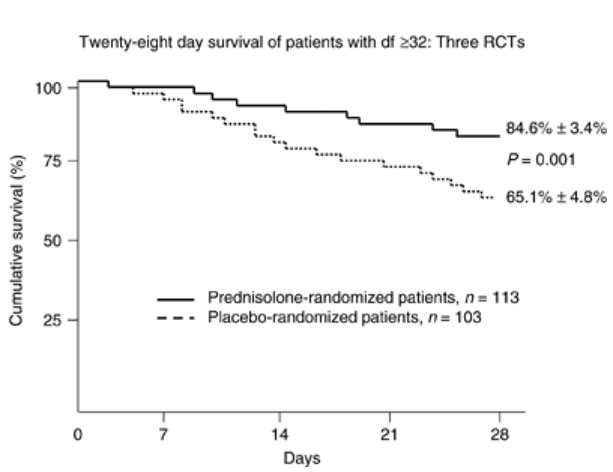
Table 32.13. Corticosteroids in Alcoholic Hepatitis—A Meta-Analysis

Trial characteristic	*N*	Risk ratio RR (95% CI)[a]	Protective efficacy (1–RR) (%)
PATIENTS WITH HEPATIC ENCEPHALOPATHY			
All trials	11	0.73 (0.58–0.92)	27
GI bleeding excluded	7	0.49 (0.33–0.72)	51
GI bleeding not excluded	4	1.06 (0.76–1.48)	NS
Quality score ≥4	7	0.56 (0.38–0.83)	44
Quality score <4	3	1.05 (0.75–1.47)	NS
"Best Estimate"[b]	5	0.64 (0.42–0.97)	36
PATIENTS WITHOUT HEPATIC ENCEPHALOPATHY			
All trials	9	1.07 (0.68–1.71)	NS
GI bleeding excluded	5	1.01 (0.36–2.81)	NS
GI bleeding not excluded	4	1.21 (0.72–2.04)	NS
Quality score ≥4	6	1.02 (0.47–2.26)	NS
Quality score <4	3	1.18 (0.65–2.13)	NS
"Best Estimate"[b]	4	1.01 (0.35–2.91)	NS

[a]If the 95% confidence interval includes unity then there is no significant therapeutic benefit (or protective efficacy) of corticosteroids for that subgroup.
[b]Best estimate = Those trials with:

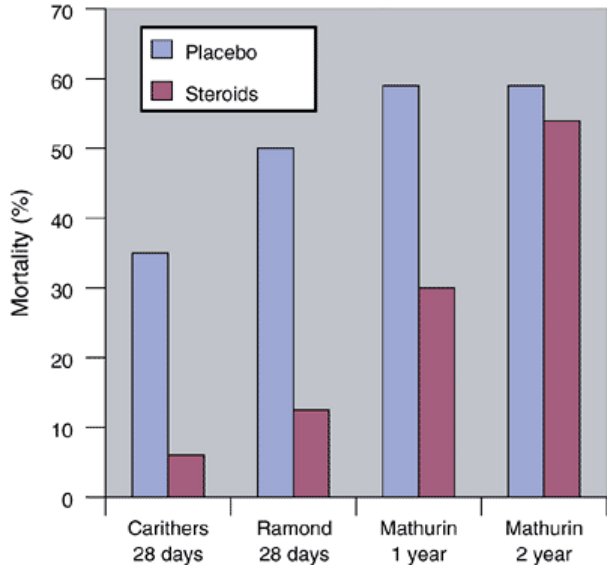
1. Quality score 4
2. Baseline equivalence between groups
3. An exclusion of active gastrointestinal bleeding.

RR, relative risk; CI, confidence interval; GI, gastrointestinal; NS, not significant; N, number of trials.



▪ **Figure 32.13** Effect of corticosteroids on the mortality of patients with alcoholic hepatitis for 28-day treatment with both prednisolone and placebo. DF, discriminant function; RCTs, randomized controlled trials.

In addition to these observations of the short-term benefits of corticosteroids in acute alcoholic hepatitis, a follow-up study showed that steroids improved the survival at 1 year but not 2 years in these patients (Fig. 32.14) (273). The long-term benefit of corticosteroids in acute alcoholic hepatitis is uncertain. A number of factors may contribute to these results and include recidivism, comorbidities, and progression of disease.



▪ **Figure 32.14** Corticosteroids showing benefit for 1-year but not for 2-year mortality in alcoholic hepatitis.

These observations provide a number of suggestions for the management of alcoholic hepatitis (330). Approximately five to seven patients need to be treated to avoid one death. This necessitates careful selection of patients to avoid the side effects of corticosteroids in the other four to six patients who will derive no clinical benefits or may suffer the adverse effects of corticosteroids. This means excluding patients with active infection and being certain of the diagnosis (liver biopsy may be necessary) because histologically confirmed alcoholic hepatitis correlates poorly with the clinical impression of alcoholic hepatitis (273). As many as 28% of patients with a clinical picture of alcoholic hepatitis do not have the histologic features of alcoholic hepatitis on liver biopsy. Only patients with severe disease (as defined by the presence of hepatic

encephalopathy, the Maddrey DF and MELD score, or the GAHS) should be treated with corticosteroids. Another recently identified marker is the early change in bilirubin content at 7 days that has been shown to accurately identify patients who are not likely to respond to corticosteroids (334). This needs to be validated before firm recommendations can be made. Finally, on the basis of pharmacologic considerations (prednisone is converted to active form—prednisolone—in the liver) rather than published data, prednisolone (40 mg daily × 4 weeks followed by a taper) should be used in favor of prednisone. One may conclude from these observations that corticosteroids reduce mortality by up to 25% in alcoholic hepatitis, but the

mortality still remains high (44%) in patients treated with corticosteroids. Therefore, other therapies or combination of therapies need to be considered (277).

Anticytokine therapy

Rationale

Cytokines are essential to the processes of hepatocyte inflammation, cell death, and regeneration. Serum levels of TNF, as well as IL-1, IL-6, and IL-8, are elevated in alcoholic hepatitis (232,335,336). Human studies have shown that the levels of soluble TNF receptor correlate linearly with an increased risk of mortality and that serum levels of TNF are high on admission and correlate with mortality (337). In addition, monocytes from patients with alcoholic hepatitis produce TNF-α at higher levels than do controls in response to endotoxin. On the basis of these observations, clinical trials have been performed using therapy targeted toward blocking TNF-α.

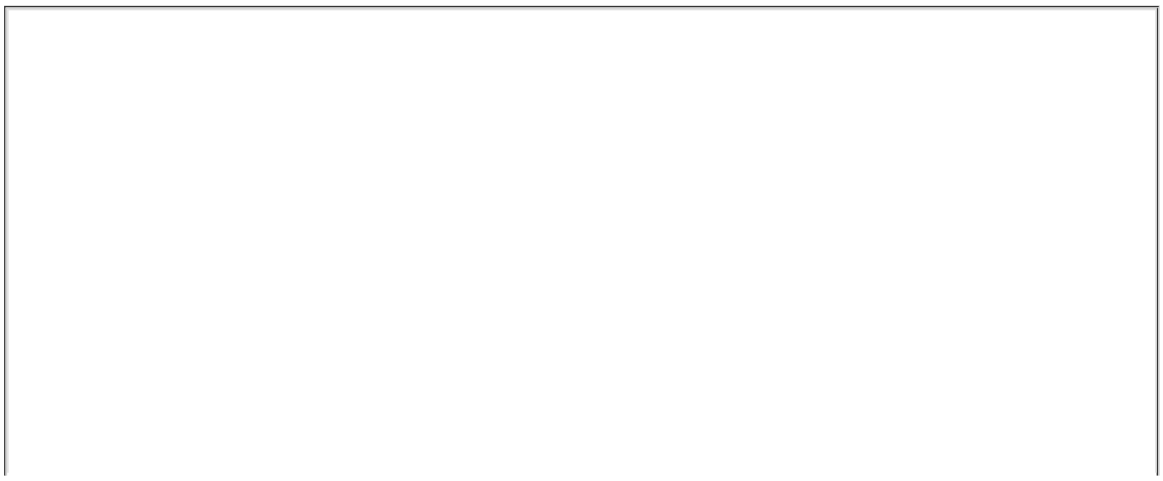
Pentoxifylline

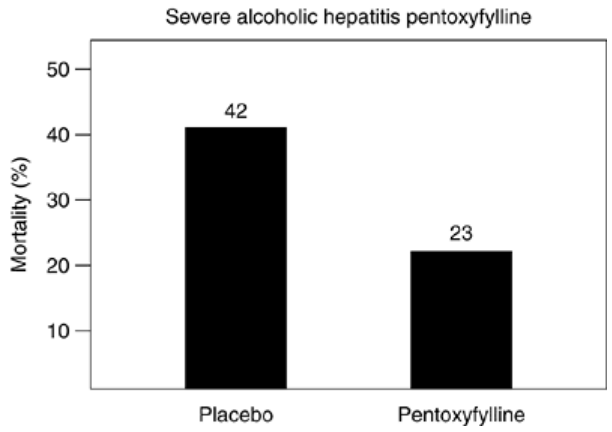
Rationale

Pentoxifylline is a phosphodiesterase inhibitor used in the treatment of peripheral vascular disease on the basis of its ability to increase erythrocyte flexibility, reduce blood viscosity, and inhibit platelet aggregation (338). Pentoxifylline also reduces the production of TNF-α, IL-5, IL-10, and IL-12 (339). It has also been shown to decrease the transcription of IL-2 and TNF-α promoters in transiently transfected normal T cells, to inhibit the activation of NFκβ and nuclear factor of activated T cells, and stimulate activation of protein-1 and cyclic adenosine monophosphate (cAMP) response of element-binding proteins. In an animal model it has been shown to reduce portal pressure in patients with cirrhosis (340).

Clinical trials

A 4-week double-blind prospective randomized trial in 101 hospitalized patients with severe alcoholic hepatitis (Maddrey score ≥32) treated with either pentoxifylline (400 mg three times a day) or placebo showed a significantly decreased mortality with pentoxifylline therapy (24% with pentoxifylline and 46% with placebo) (Fig. 32.15) (338). Hepatorenal syndrome was the major cause of death in both the groups but significantly more frequent in the placebo group. The difference in mortality between the two groups suggests a number needed to treat of 4.7, which is almost identical to the number arrived by Mathurin et al. who compared the use of steroids to placebo (273). The mechanism by which pentoxifylline decreased the development of hepatorenal syndrome is unclear but could be related to either direct effects on the liver (through any of the possible mechanisms discussed in the preceding text) or, alternatively, by a direct renal effect.





▪ **Figure 32.15** Pentoxifylline lowers mortality in acute alcoholic hepatitis compared to placebo.

Infliximab

Therapy designated to specifically target TNF-α have been used in alcoholic hepatitis.

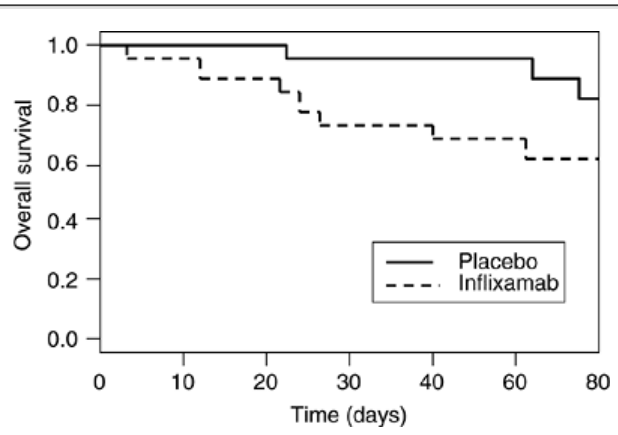
Clinical trials

Two small uncontrolled pilot studies using infliximab (IgG-1 monoclonal antibody to TNF) suggested a benefit in alcoholic hepatitis (341,342). A subsequent randomized controlled trial using infliximab (10 mg/kg) in combination with prednisolone (40 mg/day) versus prednisolone alone was begun in a total of 36 patients (343). The trial was terminated prematurely because of the significantly higher mortality related to infection in the infliximab group compared to the controls (39% vs. 11%) (Fig. 32.16). This study has been criticized on the basis of the specifics of the study design, as well as the premise for the use of such therapy (344).

Etanercept

Etanercept, a P75-soluble TNF receptor:Fc fusion protein, neutralizes soluble TNF and excludes an effect on

membrane-bound TNF. It has been used in rheumatoid and psoriatic arthritis, as well as ankylosing spondylitis (345). A single study involving 13 patients with moderate or severe alcoholic hepatitis for a 2-week duration showed that the 30-day survival rate for patients receiving etanercept was 92% (346). Adverse events (including an infection, hepatorenal decompensation, and gastrointestinal bleeding) necessitated premature discontinuation of etanercept in 23% of patients. A larger multicenter clinical trial is currently ongoing and the results of this are awaited.



▪ **Figure 32.16** Kaplan-Meier survival curve demonstrating lower survival with infliximab

compared to placebo when administered to patients with acute alcoholic hepatitis.

Finally, aggressive new therapy to remove cytokines through leukocytapheresis or other extracorporeal recirculating systems deserve further evaluation (347,348).

In summary, the results of measures directed against TNF-α appear controversial. Lack of a control arm, inclusion of patients with moderate disease (making interpretation of survival statistics uncertain), and the high dropout rate dampen the enthusiasm for the use of etanercept. On the basis of data from the studies using infliximab in alcoholic hepatitis, the extent to which complete TNF inhibition (through antibody or receptor blockade) is useful, or how best to measure it, is unclear. Further clinical trials are needed to address these issues. In addition, questions have been raised about the extent to which TNF inhibition is of benefit in this disease because TNF has been shown to be important in hepatic regeneration (233).

Antioxidants

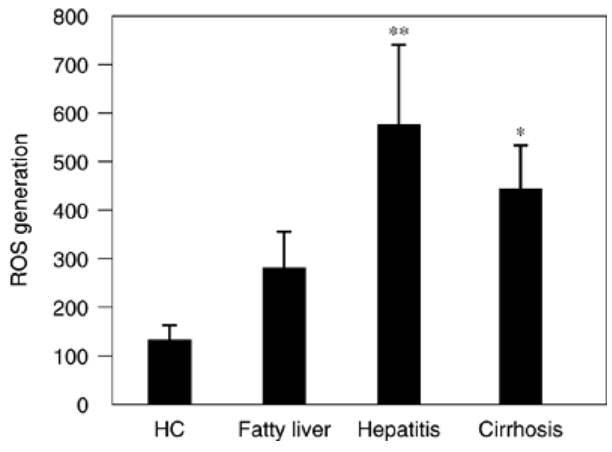
Rationale

A major mechanism of alcohol-related liver injury is mediated by the generation of superoxides, the induction of cytochrome P4502E1 activity, and the product of inducible nitric oxide synthetase–related oxidative stress (Fig. 32.17) (349,350).

In vitro studies indicate that oxidative stress sensitizes lymphocytes to TNF-α–mediated cytotoxicity that is mediated through the cellular death domain pathways (351). In addition, blood vitamin E levels and mitochondrial GSH levels are decreased by ethanol (352,353). These lead to an imbalance between alcohol-induced oxidative stress and the endogenous components of cellular defense against oxidative stress. Antioxidants also attenuate NFκβ activation and TNF-α production in monocytes of patients with alcoholic hepatitis and rat Kupffer cells in vitro (335).

Clinical trials

Vitamin E when used alone was not shown to be significantly beneficial in either alcoholic hepatitis or alcoholic cirrhosis (354,355). However, neither of these studies were optimally designed and there are data suggesting that vitamin E when combined with other antioxidants may improve outcome in alcoholic hepatitis (356).



▪ **Figure 32.17** Nonstimulated neutrophils releasing greater quantities of reactive oxygen species (ROS) in patients with alcohol-induced liver injury compared to placebo. The release of ROS is greater with increasing severity of liver injury. ** and * indicates significant difference from HC and fatty liver groups.

Nutritional supplementation

Rationale

Nutritional therapy for ALD is based on the observations of a high prevalence of protein calorie malnutrition in ALD (77,80,357). The metabolic abnormalities in ALD that contribute to nutritional deficiencies are shown in Table 32.14. Aggressive nutritional therapy should supply optimal nutritional replacement to correct preexisting malnutrition and provide sufficient amino acids to encourage hepatic regeneration without precipitating encephalopathy. The nutritional requirements in ALD are shown in Table 32.15.

Clinical trials

In a large trial of over 200 patients with ALD, 100% prevalence of nutritional deficiency was reported (358). Furthermore, protein calorie malnutrition correlated with short- and long-term mortality and the degree of liver dysfunction in patients with ALD and improved nutritional status correlated with increased survival (358). On the basis of these observations, amino acid infusions have been used in the therapy for alcoholic hepatitis (359). A total of eight randomized controlled trials have been published in 309 patients with alcoholic hepatitis, with six studies showing beneficial results and two showing negative results (360,361). The beneficial results were related to improved histology and/or liver function test results. Improved survival was only reported in a single study (362). Overall mortality in all studies combined was 17% in the treated

group and 9.6% in the control group ($P > 0.05$). In the two studies reporting a negative result, there was a large proportion of patients with inactive cirrhosis rather than active hepatitis (363,364). As is true with all artificial feedings, the enteral route is preferred over the parenteral route, if possible. On the basis of these results, one may conclude that routine protein restriction is not needed in patients with alcoholic hepatitis with or without cirrhosis even if encephalopathy is present, provided oral feeds can be tolerated. However, if encephalopathy worsens with protein-based diets, special amino acid formulations enriched in branched-chain amino acids (BCAAs) must be considered (365). A number of trials have examined various nutritional therapies for patients with alcoholic liver disease (Table 32.16). These trials demonstrate that both short-term (366,367,368,369) and long-term nutritional intervention provides therapeutic benefit for a number of important clinical outcomes (370,371,372). Nutritional therapies have also been used successfully in patients with cirrhosis undergoing liver transplantation and those with cirrhosis and HCC (374). These trials have been reviewed elsewhere (360,361). Some practical guidelines for daily dietary feeding in ALD are shown in Table 32.17.

Table 32.14. Nutritional Deficiency, Poor Intake, and Consequent Metabolic Derangements in Alcoholic Liver Disease

RELATED TO HEPATIC DYSFUNCTION
Poor intake: Anorexia, change in mental status, abdominal distension (ascites)
Malabsorption: Portal hypertensive enteropathy, bile salt deficiency
Metabolic derangement: Decreased respiratory quotient and protein synthesis; increased amino acid oxidation, resting energy expenditure
ALCOHOL-RELATED ALTERED
Energy metabolism: Anorexia, inhibition of ATP production
Protein synthesis: Increased protein breakdown and decreased synthesis
Cytokine mediated: TNF-induced apoptosis, decreased DNA synthesis, Increased energy expenditure
Micronutrient imbalances: Low thiamine, folic acid, pyridoxine, vitamin A, vitamin C, and vitamin K; hypocalcemia; hypomagnesemia; low selenium and zinc; and low choline and lecithin

ATP adenosine triphosphate; DNA, deoxyribonucleic acid.

Other agents evaluated

A number of other agents have been evaluated and these include PTU, colchicine, and anabolic steroids.

Propylthiouracil

Rationale

Alcoholic hepatitis is associated with a hypermetabolic state, with increased energy expenditure and oxygen consumption by the hepatocytes (375). This could be blocked by PTU.

Clinical trials

There have been two controlled trials of the use of short-term PTU in alcoholic hepatitis (376,377). No difference in mortality was reported in either of these studies. A recent Cochrane meta-analysis of six randomized controlled clinical trials of PTU in patients with ALD (includes steatosis, fibrosis, hepatitis, and/or cirrhosis) found no significant difference between placebo and PTU in the all-cause or liver-related mortality, complications of liver disease, or liver histology (378).

Colchicine

Rationale

Colchicine inhibits collagen production, increases hepatic collagenase activity, mobilizes ferritin deposits, and inhibits cytokine production.

Clinical trials

There have been two studies reported on the use of colchicine in the treatment of alcoholic hepatitis with no therapeutic benefit (379,380). A Cochrane review concluded that

colchicine has no role in alcoholic hepatitis outside ofcontrolled clinical trials (381).

Table 32.15. Nutritional Requirements in Alcoholic Liver Disease

Condition	Protein (g/kg/d)	Energy (Kcal/kg/d)	Energy substrate	
			%CHO	%Fat
Alcoholic hepatitis	1.0–1.5	30–40	67–80	20–33
Cirrhosis (uncomplicated)	1.0–1.5	30–40	67–80	20–33
Cirrhosis (complicated)				
Malnutrition	1.0–1.8	40–50	72	28
Cholestatis	1.0–1.5	30–40	73–80	20–27
Encephalopathy				
Grade 1 or 2	0.5–1.2	25–40	75	25
Grade 3 or 4	0.5	25–40	75	25
Liver Transplant				
Peritransplant	1.2–1.75	30–50	70–80	20–30

Post-transplant	1.0	30–35	>70	≤30

CHO, aldehyde.

Table 32.16. Nutritional Therapy in Alcoholic Liver Disease

Studies	*N*	Patient profile	Therapy	Benefit
SHORT TERM				
Kearns et al. (367)	31	Decompensated alcoholic liver disease	Hospitalized enteral feeding (isocal 1.5 g protein/kg)	Encephalopathy, serum bilirubin, antipyrine clearance
Campillo et al. (368)	26	Malnourished cirrhosis	Standard oral diet (30–35 kcal/kg daily)	Improved nutritional status and nitrogen balance
Cabré et al. (373)	35	Severely malnourished patients with cirrhosis	Hospitalized enteral feeding (2,115 kcal)	Child-Pugh score mortality (P = 0.065)
LONG TERM				
Cabré et al. (366)	71	Alcoholic cirrhosis and hepatitis	Enteral tube feeding (2,000 kcal/d) or steroids	Higher mortality due to steroids related to infection
Marchesini et al. (369)	174	Advanced cirrhosis	BCAA vs. lactalbumin/maltodextrin 14.4 g/d	Improved Child-Pugh score Trend to improved survival
Marchesini et al. (370)	64	Chronic encephalopathy	BCAA vs. casein supplements(0.24 g/kg)	Encephalopathy Nitrogen balance Bilirubin
Yoshida et	32	Symptomatic	BCAA supplements (16 g)	Delayed death

al. (371)		cirrhosis BCAA/AAA <1.0	Enteral supplements (1,000 kcal)	Less frequent hospitalization for sepsis
Hirsch et al. (372)	51 31	Decompensated Alcoholic cirrhosis Child-Pugh B and C	Oral supplements(1,000 kcal + 34 g protein) Nutritional supplement (Ensure) (1,000 kcal + 35 g protein)	Less frequent hospitalizations for infections Improved nutrition and cell-mediated immunity

Figures in parentheses are the reference number in bibliography.
BCAA, branched-chain amino acid, AAA, aromatic amino acid.

Anabolic steroids

Rationale

Anabolic steroids, testosterone, and oxandrolone have been shown to improve the hepatic histology in fatty liver and improve the synthetic functions (330). In contrast to testosterone, oxandrolone has higher nitrogen-retaining and myotrophic properties. Cotreatment with nutritional supplementation may result in further improved results.

Clinical Trials

A multicenter trial of oxandrolone in alcoholic hepatitis showed no difference in 30-day hospital mortality compared to placebo (323).

However, patients with moderate but not severe disease had improved 6-month survival. The Cochrane systematic meta-analysis of anabolic steroids in alcoholic hepatitis did not show a benefit in mortality, complications, or other outcome measures (382).

Table 32.17. Guidelines for Daily Dietary Feeding in Alcoholic Liver Disease

- Protein = 1.0–1.5 g/kg BW
- Total calories = 1.2–1.4 × REE, with a minimum of 30 kcal/kg BW
  50%–55% as carbohydrate (preferably as complex carbohydrates)
  30%–35% as fat; preferably high in unsaturated fat and with adequate essential fatty acids
- Nutrition should be given enterally by voluntary oral intake and/or by small-bore feeding tube; PPN is second choice; TPN is last choice
- Salt and water intake should be adjusted for patient's fluid volume and electrolyte status
- Liberal multivitamins and minerals
- Specialized BCAA-enriched supplements not usually necessary
  Most patients tolerate standard AA supplements
  Reserve BCAA formulations for patients who cannot tolerate the necessary amount of standard AA (which maintain nitrogen balance) without precipitating encephalopathy
  Avoid supplements providing only BCAA; they do not maintain nitrogen balance
  Conditionally essential AA and all essential AA are needed
  (Conditionally essential AA are those that can normally be synthesized from other precursors, but cannot be synthesized in patients with cirrhosis; these include choline, cystine, taurine, and tyrosine)
- Nighttime snack consisting of 500–700 cal should be given

BW, body weight; REE, resting energy expenditure; PPN peripheral parenteral nutrition; TPN, total parenteral nutrition; BCAA, branched-chain amino acids; AA, amino acids. Modified from McCullough AJ, O'Connor JF. Alcoholic liver disease: proposed recommendations for the American college of Gastroenterology. *Am J Gastroenterol* 1998;93(11):2022–2036.

We conclude that these agents have a secondary role as an experimental option in alcoholic hepatitis.

Long-Term Management of Alcoholic Liver Disease

Although less acute than alcoholic hepatitis, continuous long-term management is an important component of the treatment of patients with ALD.

Nutritional therapy

Protein calorie malnutrition that is widely prevalent in ALD (358) is associated with major complications observed in cirrhosis (e.g., infection, encephalopathy, and ascites) and indicates a poor prognosis. Consequently, the importance of the clinician recognizing and understanding the significance of malnutrition in these patients has been emphasized.

Enteral feeding for 3 to 4 weeks in hospitalized, severely malnourished or decompensated patients with cirrhosis improved survival ($P < 0.065$), hepatic encephalopathy, liver function, and Child-Pugh score, as compared with controls receiving a standard oral diet (374). In longer-term studies, Marchesini et al. compared equinitrogenous amounts of dietary BCAA versus casein supplements for 3 to 6 months in patients with chronic hepatic encephalopathy (370). BCAA significantly improved response to encephalopathy, nitrogen balance, and serum bilirubin compared with casein. Hirsch et al. supplemented 34 g of protein and 1,000 kcal to a regular diet in decompensated alcoholic patients with cirrhosis and it reduced hospitalizations for infections over a 1-year period (372). These studies are important because they emphasize the concept that patient selection and long-term therapy may be important factors for employing and demonstrating the benefits of nutritional therapy. Because standard nutritional supplements are effective and the cost of BCAA is high, the use of BCAA-enriched formulations should be restricted to patients who cannot tolerate nutritionally required amounts of standard formulations.

Changes in the dietary feeding patterns may also be beneficial. After an overnight fast, patients with cirrhosis obtain more than 70% of nonprotein calories from fat as compared with 40% in normal volunteers (383). Therefore, patients with alcoholic cirrhosis have early recruitment of alternative fuels and have metabolic profiles similar to those observed with prolonged fasting. Consequently, it has been demonstrated that patients with cirrhosis should not be allowed to starve for extended periods and deserve frequent interval feedings, emphasizing a nighttime snack and morning feeding that improve nitrogen balance (384,385,386).

It now seems clear that long-term aggressive nutritional therapy is necessary and reasonable for these patients. General goals and practical points for nutritional therapy in chronic liver disease (Tables 32.15 and 32.17) have been suggested, but three additional points need to be emphasized (361,387). First, nutritional assessment should be an ongoing process. Second, multiple feedings emphasizing breakfast and a nighttime snack with a regular oral diet at higher than usual dietary intakes (1.2 to 1.5 g/kg for protein and 35 to 40 kcal/kg for energy) seem to be indicated (384,385,386). Third, during intermittent acute illness or exacerbations of the underlying chronic liver disease, a higher than normal protein intake (1.5 to 2.0 g/kg body weight and 40 to 45 kcal/kg energy) improves protein calorie malnutrition (361,383,387).

Propylthiouracil

The hypermetabolic state induced by alcohol ingestion can be ameliorated by PTU (375). Direct vasodilator effects of PTU and its antioxidant potential have been suggested to be of value in

alcoholic cirrhosis.

Clinical trials

In a large prospective study, involving 310 patients, therapy with PTU for up to 2 years showed improved survival in the PTU-treated group compared to placebo (388). A Cochrane review of PTU in ALD reviewed six randomized controlled trials in a total of 710 patients administered PTU or placebo. There was no significant benefit of PTU compared to placebo on the total or liver-related mortality, complications of liver disease, or liver histology. There was an increase in nonserious adverse effects that was not significant (378).

S-Adenosyl L-Methionine

Rationale

SAMe is produced from methionine and ATP in the presence of the enzyme SAMe synthetase also known as *MAT* (389). SAMe is the principal biologic methyl donor involved in transmethylation, trans-sulfuration, and aminopropylation. The trans-sulfuration pathway is responsible for GSH synthesis—the main cellular antioxidant. Impaired MAT activity occurs in cirrhosis, resulting in impaired methionine metabolism with increased levels of methionine and homocysteine (255). Deficient MAT results in low SAMe levels and hence low GSH levels. Exogenous GSH is not useful therapeutically because it does not penetrate the hepatocytes. Cysteine is the precursor of GSH and

methionine is the precursor of cysteine. Cysteine and methionine administration have been unsuccessful in correcting the deficiency of GSH. Oral administration of SAMe bypasses the deficit in SAMe synthesis from methionine.

Clinical trials

Oral SAMe administration has been shown to be effective in humans studies in ALD after a few initial disappointing results (389,390). SAMe supplementation improved survival in patients with alcoholic cirrhosis who were Child-Pugh class A and B (390). In contrast, the Cochrane database, based on eight randomized controlled trials with 330 patients in different stages of ALD, did not demonstrate any significant effect of SAMe on mortality, liver-related mortality, complications, or liver transplantation in patients with ALD (391). It was concluded that SAMe should not be used for ALD outside randomized clinical trials.

Colchicine

Colchicine has been used in alcoholic cirrhosis, with beneficial results reported, but few hepatologists prescribe this agent. This may be related to doubts cast on the results of the biggest study reported on the use of colchicine in cirrhosis (392), its potential toxicity, and the need for long-term use before any potential benefits can be observed. A systematic meta-analysis by the Cochrane group showed that a total of 14 randomized trials with 1,150 patients have been studied (patients with alcoholic fibrosis, alcoholic hepatitis, and/or alcoholic cirrhosis, as well as patients with viral-induced or cryptogenic fibrosis and/or cirrhosis) (381). The results showed that there was no benefit on overall mortality, liver-related mortality, liver function tests, or histology, with an increased risk of adverse effects related to colchicine therapy. On the basis of the currently available evidence, colchicine cannot be recommended for the treatment of established alcoholic cirrhosis.

Antiviral therapy

Alcohol use even in moderate quantities has been suggested to worsen the course of hepatitis C (135,303). Additionally, it has been shown that the response rates to interferon-based treatment protocols are less effective in the presence of active alcohol use (393). It is therefore recommended that therapy for hepatitis C be started only in patients who become abstinent. If hepatitis C can be eradicated, the long-term prognosis of ALD should improve.

Liver transplantation for alcoholic liver disease

ALD is the second most common indication for orthotopic liver transplantation (OLT) for chronic liver disease in the Western world (394). In the United States, although specific candidate selection criteria vary from center to center, patients with ALD undergo transplantation in virtually all centers (395). There is resistance to performing transplantation in patients with ALD because of

the pretransplantation socioethical issues of donating organs to patients who have self-inflicted liver disease while others who did not pursue such an active self-injurious course wait and potentially die (396,397). Other concerns include questions about the patient's ability to comply with the complex clinical protocols and immunosuppressive regimens after the transplantation, the rate of recidivism to a behavior of alcohol abuse, and recurrence of injury to the transplanted liver (294). As the number of OLT in ALD increases, it is becoming acceptable to perform transplantation in these patients because their outcome after OLT is similar to that in nonalcoholic patients (397,398).

A 6-month period of abstinence has been recommended as a minimal listing criterion, allowing chemical dependency issues to be resolved during this period. Adherence to the 6-month abstinence could result in a resolution of the anti-inflammatory effects of recent alcohol consumption and, therefore, may make OLT unnecessary in a subset of these patients. The requirement for a fixed abstinence period, the so-called 6-month rule, as a predictor of future abstinence is arbitrary but used most often. However, many abstinence experts are skeptical about this rule because it ignores the complex nature of addictive behavior and does not accurately predict future drinking by alcoholic candidates for liver transplantation (398).

An evaluation of the peritransplantation period in ALD showed that patients were more ill at the time of OLT and likely to have prolonged intensive care unit stays and increased blood product requirements (399). Decreased acute and chronic rejection after OLT have been reported in patients with ALD compared to nonalcoholic subjects and is probably related to the depression in cellular immunity from the alcohol abuse before OLT (400,401,402). There have been a total of 22 published reports on the outcome of liver transplantation in alcoholic patients in over 1,200 patients followed up over 11 years (Table 32.18) (294,394,399,403,404,405,406,407,408,409,410,411,412,413,414,415,416,417,418,419,420,421).

One third to one half of the transplant recipients with ALD will report some use of alcohol in the first 5 years after transplantation (397). It has been suggested that the consequences for alcohol is minimal for many recipients because the amount consumed is small and occurs infrequently, but there is little reliable data to support this contention. Rates of recidivism at 3 to 5 years after OLT have been reported between 11% and 49% (286,294,408,409,415,421,422). It should be emphasized that the rate of recidivism depends on the method used to assess alcohol use and abuse. Higher rates have been reported with objective criteria such as urine and blood alcohol levels, CDT, or a detailed structured

interview rather than unstructured questioning of the patient (423,424). However, recipients who relapse into alcohol use after OLT have similar or potentially lower graft failure rates compared to non-ALD patients. Poor follow-up attendance and noncompliance with therapy is observed in only a minority of patients; graft rejection rates are similar for patients with ALD (41% acute rejection and 5.6% chronic rejection) compared to the non-ALD patients (43.7% acute rejection and 6.2% chronic rejection) (394,397,425). Therefore, OLT should be offered to appropriately selected patients with ALD.

Table 32.18. Results of Liver Transplantation for Alcoholic Liver Disease

Author	Number of patients	1-year survival	5-year survival	Recidivism (%)
Mackie et al. (294)	64	49 (2 y)	—	45
Burra and Lucey (394)	51	—	64	33
Bellamy et al. (399)	123	84	72	19.7
Gish et al. (403)	27	93	—	21
Stefanini et al. (404)	18	73 (6 m)	—	27.2

Zibari et al. (405)	42	74	71 (3 y)	7
Howard and Fahy (406)	40	79	—	95
Lucey et al. (407)	59	80	77	34
Osorio et al. (408)	43	100	—	19
Pageaux et al. (409)	53	—	—	32
Goldar-Najafi et al. (410)	56	36 (1 y)		9
Jain et al. (411)	185	—	72	20
Pereira and Williams (412)	56	—	—	8.4
Dhar et al. (413)	35	74 (2 y)	—	—
Tang et al. (414)	56	—	—	50
Fabrega et al. (415)	44	—	—	18
Kumar et al. (416)	83	74	—	12
Bird et al. (417)	24	66	—	17
Knechtle et al. (418)	41	83	71	13
Platz et al. (419)	167	—	96.8	17.1
Gerhardt et al. (420)	41	—	—	8.2
Berlakovichet al. (421)	80	67	49	12.8

Figures in parentheses under the column “authors” are the corresponding reference numbers in the reference section.

One of the issues that is still unresolved is the role of OLT in acute alcoholic hepatitis. It was observed in a previous study using retrospective histologic analysis of the explanted liver that superimposed alcoholic hepatitis did not worsen the outcome after OLT. However, all patients in this study had been abstinent for at least 3 months, had a DF less than 32, and had variable clinical and biologic features of alcoholic hepatitis at the time of OLT. Therefore, the role of OLT in acute alcoholic hepatitis is unresolved at this time (284). With the availability of living donor transplantation and extended criteria donor, this is likely to further the debate on this issue.

Potential New Treatment Options

Various nonconventional treatment options have been considered promising in the therapy of ALD. Recently, advances in the understanding of the pathogenesis of ALD have suggested new targets for more successful intervention. Some of the nontraditional treatment options include the following.

Silymarin: Silymarin (milk thistle) is a mixture of flavinolignans (i.e., silibinin, silidianin, silichristin, and silybin), of which silybin is the most active (426). The exact mechanism of action of silymarin is not yet clear but is most likely related to its antioxidant properties.

There have been six published trials of silymarin in patients with ALD in doses ranging from 80 to 420 mg/day for periods of 4 weeks to 6 years, with a total of 302 patients treated with silymarin and 305 patients with placebo (426). Normalization of liver function tests, improvement in liver histology, and possibly survival was observed in a significantly higher proportion of patients treated with silymarin compared to placebo. No adverse effects were reported. A Cochrane systematic review and meta-analysis of the 13 published studies of silymarin in ALD and hepatitis B- or C-related liver diseases showed that the overall methodologic quality of the studies was low and based on high-quality trials and that milk thistle does not significantly influence the course of patients with alcoholic and/or hepatitis B or C liver diseases (427).

Other agents: Thalidomide, misoprostol, adiponectin, and probiotics have been shown in preliminary reports to have anticytokine properties (344,428,429,430). However, it must be emphasized that in the past many promising treatments have not passed the test of time as treatment for ALD and alcoholic hepatitis. Emerging data suggest a role for TNF-α–mediated apoptosis in

alcoholic hepatitis, and therapy targeting this cytokine to inhibit apoptosis may be effective (431).

Future Directions

In light of a still incomplete understanding of the involved pathophysiologic mechanisms and conflicting data about the efficacy of specific interventional therapies of ALD, a conservative approach seems justified. These include general supportive care, aggressive nutritional interventions, and the judicious use of corticosteroids and pentoxifylline in select patients with severe alcoholic hepatitis. In addition, abstinence from alcohol, treating the comorbidities of obesity and hepatitis C, and continuous nutritional monitoring are prudent on a long-term basis, with liver transplantation offered for select patients with progressive disease. Algorithms have been proposed for both short-term and long-term management of ALD (Tables 32.19 and 32.20). Although these algorithms are by necessity incomplete, they are provided to stimulate different approaches to this disease and to emphasize several of the following important issues:

1. Abstinence and dietary manipulation should be emphasized in the long-term management of these patients. The issue of abstinence from alcohol will play an important role in determining medical treatment, as well as candidacy for liver transplantation.
2. The high prevalence of hepatitis C must be considered with respect to its potential influence on prognosis and therapeutic strategies.
3. Future therapeutic directions should aim at specific pathophysiologic mechanisms of alcohol-induced hepatocellular damage, as well as the regulation of hepatic regeneration in this disease. Combination therapy targeting both direct and indirect mechanisms of alcohol-induced hepatocellular damage, as well as the regenerative capacity of the injured liver, may prove to be necessary after assessment of disease

severity and prognosis. Clinical trials of combined therapy are needed and include the following:

   a. A mechanism to reduce hypermetabolism and centrilobular hypoxia/ischemia
   b. A means to maintain or enhance gut integrity; the role of nutrition needs to be investigated; furthermore, more objective and systemic ways to assess the function and integrity of the intestinal mucosa are required
   c. The role of bowel sterilization with antibiotics to decrease bacterial translocation and endotoxin- and cytokine-mediated liver damage

d. Modification of immunologic mechanisms

e. Alteration of cytokine-mediated inflammation and fibrosis; potential therapeutic interventions include antibodies or antagonists to specific cytokines of pathogenetic importance and drugs that modulate the actions of these cytokines; therapies directed against anti-TNF may be particularly helpful

f. Inhibition of fibrogenesis by blocking factors that promote fibrogenesis and by stimulating collagen degradation or suppressing collagen gene expression

g. Hepatotropic agents to maximize the capacity of cell repair

h. Exploration of new strategies to decrease lipid peroxidation and its sequelae; SAMe may be particularly relevant in this area

i. Orthotopic and living donor liver transplantation, which are a realistic option for alcoholic cirrhosis; their role in alcoholic hepatitis remains a matter of debate

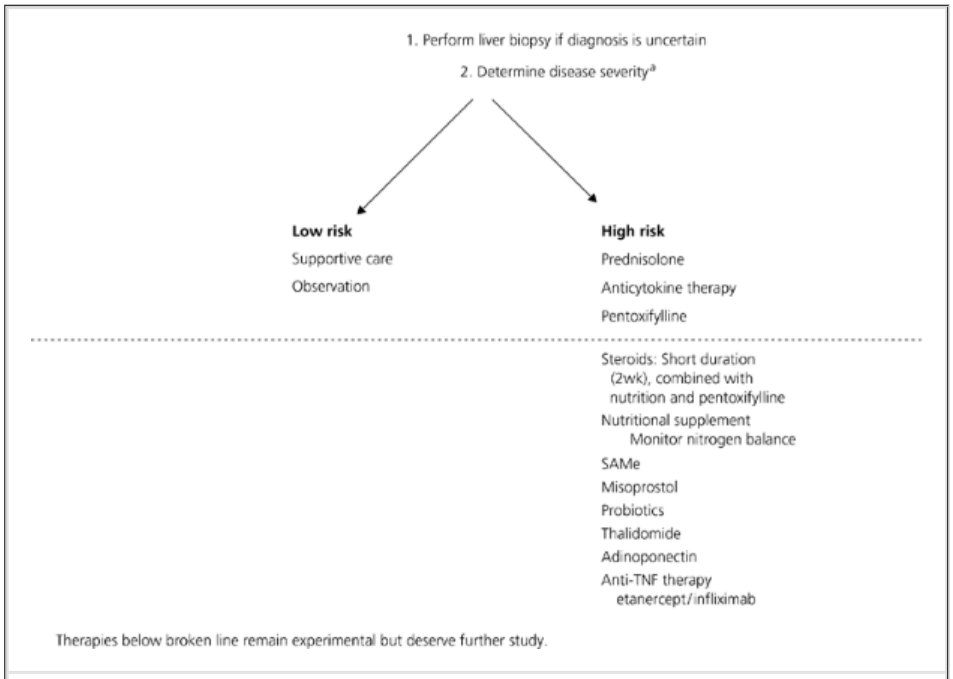


Table 32.19. A Proposed Therapeutic Algorithm for Alcoholic Hepatitis

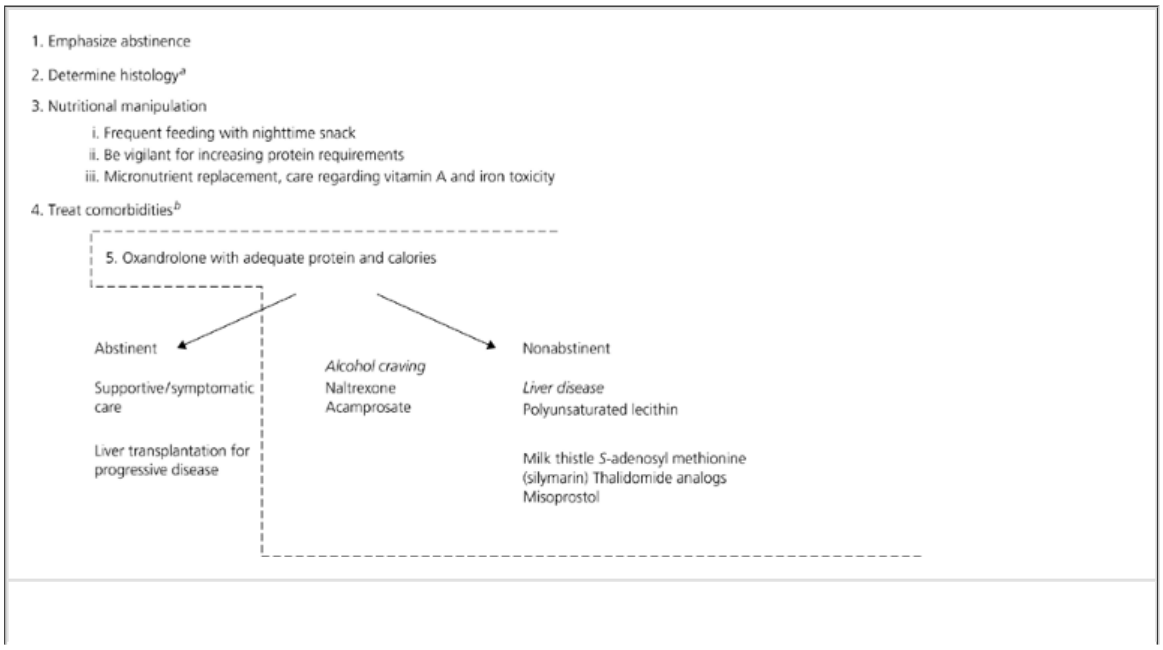


Table 32.20. Proposed Therapeutic Algorithm for the Long-Term Management of Alcoholic Liver Disease

Summary and Conclusions

ALD is a common illness, which develops in only a subgroup of people who chronically use or abuse alcohol. The hepatotoxic dose of alcohol is nonuniform and dependent on incompletely understood risk factors, which include gender, ethnicity, hepatitis C, nutritional status (particularly obesity), and possibly genetic factors. These factors, in addition to a variable natural history and vaguely defined epidemiology and pathophysiology, can make ALD difficult to diagnose and frustrating to manage.

The management of the complications of chronic liver disease (e.g., ascites, portal hypertension associated bleeding, encephalopathy, and hepatocellular carcinoma) is similar in alcoholic and non-ALD patients. Abstinence remains the cornerstone of therapy for ALD. There is also consensus for the use of corticosteroids and pentoxifylline in severe alcoholic hepatitis for maintaining good nutritional status, for treating comorbidities in all forms of ALD, and for liver transplantation in carefully selected patients with end-stage ALD. No other therapies can be recommended at the present time, although nascent data suggest that a number of newer therapies may be effective, as discussed in the preceding text.

Building on our increased knowledge of the molecular basis of ALD, a greater understanding of the pathophysiology of ALD and the interactive role of other cofactors in causing hepatotoxicity needs to remain a major focus of alcohol research.

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Chapter 33
Drug-Induced Liver Disease

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Key Concepts

- Over 300 drugs in current use have been implicated in causing liver injury, but there is strong evidence for causality with fewer than 20 of these agents.
- Most implicated drugs cause liver disease in fewer than 1 in 10,000 persons who are exposed, but the frequency of hepatotoxicity is influenced by genetic factors, age, gender, intake of other drugs or alcohol, nutritional status, and preexisting liver disease.
- Interactions between drugs and disease-related factors include highly active anti-retroviral treatment (HAART) and hepatitis C virus (HCV) infection, tamoxifen and nonalcoholic fatty liver disease (NAFLD), methotrexate with diabetes and hepatic fibrosis, and antituberculosis drugs with chronic hepatitis B and hepatitis C.
- The clinicopathologic spectrum of drug-induced liver disease ranges from nonspecific injury to acute and chronic hepatitis, granulomatous hepatitis, cholestatic reactions, vascular lesions, and hepatic tumors.
- Although characteristic "signature" patterns are observed with some drugs, others are associated with diverse clinical syndromes.
- Although the liver biochemistry profile may aid initial evaluation, liver biopsy remains the gold standard for defining the type and extent of drug-induced liver disease.
- Pathogenic mechanisms underlying hepatotoxicity include dose-dependent injury, metabolic idiosyncrasy, and immunoallergic reactions. The latter may be part of the reactive metabolite syndrome, a multisystem disorder with hallmarks of hypersensitivity.
- Supportive measures remain the cornerstone in managing patients with drug-induced liver disease. Early recognition of drug toxicity and immediate withdrawal of the offending drug are critical. With the exception of *N*-acetylcysteine, there are no specific antidotes. Corticosteroids are not routinely recommended but may be valuable in select cases showing pronounced hypersensitivity characteristics (e.g., allopurinol). Anecdotal evidence favors the use of ursodeoxycholic acid in the setting of protracted cholestasis. Early consultation with a liver transplantation center is mandatory for individuals developing progressive impairment of liver function.
- Serial liver test profiles are often recommended to facilitate early detection of liver injury. However, with few exceptions, the sensitivity and cost-effectiveness of this approach remains untested.

- Clinical evaluation of symptoms that could be drug related is critical in facilitating early detection, and subsequent drug discontinuation is the key to preventing adverse outcomes.
- Herbal hepatotoxicity and use of recreational drugs (of abuse) should now be considered in the differential diagnosis of all liver disorders.

Introduction

Terminology and Definitions

Hepatotoxicity is liver injury caused by foreign compounds (*xenobiotics*). These include prescribed and nonprescribed therapeutic agents (*drugs*), including herbal medicines, and a vast array of other organic and inorganic substances that may be ingested deliberately or accidentally and that may contaminate the environment, workplace, or home. Therefore, xenobiotics include pesticides, herbicides, plant, fungal, and microbial products, each of which may have toxic and/or carcinogenic properties. The present chapter concentrates on *drug-induced liver injury*, in which hepatotoxicity is caused by drugs used either in medical practice or those used by individuals for therapeutic, nutritional, or recreational purposes; the latter including drugs of abuse. Although passing reference is made to other hepatotoxins, the interested reader is referred to more detailed texts for a comprehensive coverage of environmental and industrial hepatotoxicity (1,2).

Injury to the liver is largely defined by increased blood levels of proteins that are liberated from damaged hepatocytes; a typical example is alanine aminotransferase (ALT). To implicate a drug as the cause of ALT level elevation we need to know that (a) there is no other hepatic process that could account for the test abnormality (e.g., steatosis), (b) the logistics (particularly the temporal relationships) relating drug intake

and liver test abnormality are consistent and compelling, and (c) elevation of ALT level really means that the liver is injured. There is often a considerable amount of uncertainty in each of these three areas; some practical implications will be dealt with in "Hepatic Adaptation" (physiologic responses by the liver when exposed to drugs) and "Diagnosis".

We have avoided the term *hepatic dysfunction* in this text because of its confusion between injury, adaptation, and indices that truly reflect the functions of the liver. Likewise, the pathologically meaningless term *transaminitis* (an ALT level elevation without histologic evidence of liver inflammation or cell damage) is not used. When the presence of a major (fivefold or greater) elevation in the level of ALT clearly indicates liver injury, or when one or more of the *functions of the liver* are abnormal (e.g., low levels of plasma proteins such as albumin and clotting factors synthesized by the liver or clinicopathologic evidence of impaired bile flow [*cholestasis*]), it is *highly probable* that a drug to which the person has been exposed is the cause of liver disease. Ideally, however, definition of *drug-induced liver disease* requires histopathologic characterization rather than syndromic recognition of liver test abnormalities. Although the distinction between injury and disease is sometimes artificial because they clearly overlap, it is maintained, wherever possible, in this chapter so as to provide insights into the clinical significance of hepatic adverse drug reactions.

As new drugs emerge, the evidence that they induce liver injury is often weak and circumstantial; it is sometimes hotly debated because of the implications for further use and marketing of valuable therapeutic agents or for medicolegal implications (3). To partly meet the challenge of possible newer types of drug-induced liver disease that are not yet well defined as reproducible entities, we have included a section "Emerging Drugs" at the end of this chapter. Another definitional challenge is that drugs may sometimes alter physiologic parameters that have an impact on hepatic viability. In particular, some drugs can profoundly reduce hepatic blood flow and oxygen delivery, induce hyperthermia, or modify arterial supply to major bile ducts, each of which can result in liver injury that may be minor or profound. Cocaine, general anesthetic agents, alcohol, intra-arterial floxuridine, and "ecstasy" are agents that most likely produce liver injury by such indirect mechanisms rather than by direct hepatotoxicity or *adverse drug reactions*.

Another challenge in defining drug-induced liver disease is the increasing number of circumstances in which drug ingestion appears to contribute to chronic liver disease or hepatic tumors, as discussed in later sections. The lead time ("latent period") to onset or diagnosis of such associations is many months or years. As a result, repeated observations and case–control studies are essential to ensure that they are not chance associations, while experimental evidence from laboratory or animal studies are desirable to invoke biologically plausible mechanistic explanations for this type of effect of the drug on the liver.

Therefore, there is no "gold standard" by which drugs can be proved to have a unique etiologic role in liver disease, particularly because some of the disorders with which they are associated appear very similar or identical to syndromes associated with other causes.

Further, it is increasingly apparent that drugs may interact with each other or with other hepatotoxins (particularly alcohol) as part of *interactive hepatotoxicity*, as well as with viruses (human immunodeficiency virus [HIV] and hepatitis B and C viruses), immune mechanisms (HIV/acquired immunodeficiency syndrome [AIDS] and bone marrow transplantation), and metabolic factors (nonalcoholic steatohepatitis [NASH]), to accentuate or cause liver injury. Such interactions may be exceedingly difficult to recognize or prove, and nearly impossible to quantify as "relative risk" by conventional epidemiologic techniques. The evidence implicating involvement of drugs in liver injury associated with more complex medical settings is discussed in a later section.

Dose-Dependent Hepatotoxicity and Hepatic Drug Reactions

Some agents possess a high degree of *intrinsic hepatotoxic potential*; they cause *dose-dependent liver injury* in humans and usually many other species. The history of industrial hepatotoxicity is replete with such examples, among which dimethylnitrosamine, carbon tetrachloride, tetrachloroethane, trinitrotoluene, phosphorus, tannic acid, and vinyl chloride are well known. Some early therapeutic drugs and anesthetic agents (arsenicals, chloroform) have also been assigned to this category, although older studies are inadequate in ascertaining causal mechanisms because viral hepatitis, hepatic perfusion, and tissue oxygenation could not be assessed by contemporary criteria. Among today's drugs, very few are *dose-dependent hepatotoxins* (Table 33.1). For those that are, as illustrated by acetaminophen, it is not the chemical structure of the parent drug that is responsible for liver injury but rather the production of chemically reactive metabolites that interfere with the integrity of the liver. It follows that for most dose-dependent hepatotoxins, a range of host factors predicate the amount of reactive (toxic) metabolites that accumulate, thereby determining the risk of liver injury for a given dose of the toxicant.

The vast majority (>95%) of drugs implicated as causing drug-induced liver disease are clearly *not* dose-dependent human hepatotoxins, although some of them (usually at high, *nonpharmacologic* doses) produce experimental liver injury. This relative "intrinsic hepatotoxic potential" tends to be roughly proportional to the risk of liver injury in humans (2); for a few agents there is also evidence that liver injury is partially dose dependent (e.g., perhexiline maleate, herbal medicines, tacrine, dantrolene, cyclophosphamide, sex steroids, and cyclosporine). However, the frequency with which such agents cause liver injury among those exposed is either low or extremely small, ranging from 0.5% to 2% with chlorpromazine and isoniazid (INH), through more typical rates of 1 to 10 cases per 100,000 persons exposed, to even lower rates (e.g., 1 case per 1,000,000 persons exposed) with minocycline, and some of the oxypenicillins and nonsteroidal anti-

inflammatory drugs (NSAIDs) (Table 33.2).

Table 33.1. Examples of Dose-Dependent Hepatotoxins

- Acetaminophen (paracetamol)
- Drugs used in cancer chemotherapy (especially those used with radiotherapy); cyclophosphamide, busulphan, bis-chlorethyl-nitrosourea
- Amodiaquine
- Hycanthone
- Carbon tetrachloride, dimethylnitrosamine, methylenedianiline
- Plant and fungal toxins: Pyrrolizidine alkaloids, aflatoxin
- Ethanol
- Metals: Copper, iron, mercury
- Bile salts

It is clear that for these rare, dose-independent, unpredictable, or *idiosyncratic drug reactions* to occur, it is the host response to the drug that often determines liver injury, not the dose or chemical structures of the agent and its metabolites. Idiosyncratic hepatotoxicity is difficult to reproduce in other species, and it is therefore hard to ascertain the pathogenetic mechanisms involved. Clinical recognition will always remain a challenge because most doctors will never encounter individual reactions or observe them more often than once or twice in a professional lifetime.

Two broad types of pathogenic mechanisms could account for idiosyncratic hepatic drug reactions. The first is "metabolic idiosyncrasy," in which pathways of drug metabolism or disposition favor drug accumulation or formation of toxic metabolites. The underlying determinants include pharmacogenetic variability of drug metabolism and expression of "antistress" and antioxidant cell defense pathways. The metabolic

idiosyncrasy concept now needs to be extended to consider ways in which the liver, a highly adaptable organ, normally counters the "stress" of potentially damaging chemicals. Therefore, using experimental toxicants, it has become evident that foreign compounds, drug metabolites, and resultant oxidative stress can stimulate or interrupt intracellular signaling pathways that converge on the target genes involved in stress responses, cell death, or cell cycle regulation. Alternatively, they may directly activate transcriptional regulators to the same effect or more indirectly interfere with cell integrity and cell death pathways by their interactions with mitochondrial function and integrity; the latter includes the unique "guardian role" of mitochondria for cell survival, as well as in energy generation. As discussed in Chapter 8, drugs can alter the regulation of adenosine triphosphate (ATP)-dependent transporters that actively pump drug metabolites out of liver cells, particularly through canalicular pathways that are physiologically engaged in the generation of bile. Finally, drugs, reactive metabolites, and oxidative stress can interact with the cytoskeletal determinants of cellular transport, receptor signaling, and cell–cell communication (discussed further in Chapter 34).

Table 33.2. Frequencies of Some Types of Idiosyncratic Drug-Induced Liver Diseases

Frequency[a]	Drugs
5–20/1,000 exposed	Isoniazid, chlorpromazine, dantrolene
1–2.5/10,000 exposed	Estrogen-induced cholestasis
0.5–20/10,000 exposed	Ketoconazole
1–10/100,000 exposed	Diclofenac, sulindac, phenytoin, flucloxacillin
0.5–3/100,000 exposed	Amoxicillin–clavulanate, nitrofurantoin, terbinafine, dicloxacillin
≤1–10/1,000,000 exposed	Minocycline

[a]Based on published data referred to in the text.

The alternative pathogenic mechanism underlying idiosyncratic hepatic drug reactions is an "immunoallergic" response. This refers to classical "hypersensitivity" in the sense that repeated exposure results in an

exaggerated and unhelpful tissue-based or systemic injurious inflammatory response. Immunoallergic reactions are even less well understood than metabolic idiosyncrasy for their role in drug-induced liver disease. The possible immunologic mechanisms are reviewed elsewhere (4,5). Some may be examples of drug-induced autoimmunity in which the liver is the principal organ involved, implying that the drug (or its metabolites) induces immune dysregulation (6). Syndromes of chronic hepatitis with hyperglobulinemia and autoantibodies (e.g., with nitrofurantoin, minocycline or diclofenac) are examples of such an immune-based mechanism. There is increasing evidence that, for a subset of reactions, reactive metabolites are involved in recruiting inflammatory mechanisms, either as haptens or with molecular mimicry (7). Regulation of hepatic cytokines may also be important, as shown by studies of drug hepatotoxicity in mice with targeted disruptions of interleukin (IL)-4, IL-10, or cyclo-oxygenase 2 (COX-2) mediators that help prevent allergic hepatitis (5). Recruitment of eosinophils to the liver in the later stages of idiosyncratic drug reactions may depend on the expression of the chemokine, eotaxin (8).

It is often not possible to clearly distinguish between these apparently diametrically different causative mechanisms. They may often overlap, particularly because hepatic inflammatory reactions appear to evoke the liver's own innate immunity, as seen by the presence of liver lymphocytes and activated Kupffer cells (resident macrophages). Ultimately, it is most likely that the principal factors predicating idiosyncratic drug reactions are *genetically determined* (4,9,10,11,12,13); this provides a challenge for future studies directed at prevention and for timely interventions to avoid the adverse clinical outcomes that are unacceptably common for some agents and reactions (see "Improving Outcomes").

The Importance of Drug-Induced Liver Disease

The importance of drug-induced liver disease is summarized in Table 33.3. The pertinent factors are the disproportionate importance and potential preventability of serious hepatic adverse drug reactions in older

people. Some newer aspects include the potential of drugs to produce synergistic hepatotoxicity in persons with, for example, viral hepatitis, HIV/AIDS, and NASH; this is discussed later in relation to interactive hepatotoxicity and interactions with alcohol. The low frequency of reactions for a host of commonly used drugs can delay recognition of drug-induced liver disease; the resultant continued ingestion of the causative agent is the single most important determinant of adverse outcome. In addition to the key responsibility of physicians to make an early diagnosis and stop patients' exposure to potentially implicated agents, they have moral, ethical, and medicolegal responsibilities to prevent, mitigate, and report iatrogenic disease.

Table 33.3. Factors that Contribute to the Importance of Drug-Induced Liver Disease

- Approximately 6% of all adverse drug reactions
- Higher frequency among severe adverse drug reactions
- Most frequent cause for postmarketing withdrawal of medications
- Preventable or correctable cause of acute and chronic liver disease
- Approximately 5% of cases of jaundice or acute hepatitis in the community
- Higher proportion (10%–40% depending on age) of cases of hepatitis admitted to hospital
- Important cause of acute liver failure (>50% of cases in the United States; 36% from acetaminophen, 16% idiosyncratic hepatic drug reactions)
- Common cause of undiagnosed liver injury, particularly among persons aged >50 years.
- More than 300 currently used drugs cited in literature as potential causes
- Low frequency of liver injury leads to cases often being overlooked and difficulty in attributing causality
- One agent may cause more than one pattern of drug-induced liver disease
- Critical importance of early diagnosis and stopping drug exposure to avoid progression and poor outcomes
- Poor understanding of pathogenic mechanisms makes reactions difficult to predict and prevent
- Role of drugs in synergistic hepatotoxicity with viral hepatitis, nonalcoholic steatohepatitis, human immunodeficiency virus/acquired immunodeficiency syndrome, and bone marrow or organ transplantation
- Moral/ethical responsibility to prevent or minimize iatrogenic disease
- Medicolegal implications of this responsibility (informed consent, practice standards, due diligence, etc.)

The increasing number of pharmacologic agents for which adverse hepatic drug reactions have been described is a major challenge for clinicians (2,9,14,15,16,17,18,19,20). In addition to case recognition, this invokes consideration of what level of patient information should be regarded as appropriate at the time of prescribing drugs, and how this information should be imparted to the consumer.

Diversity of Clinical Expression

Drugs have become the greater mimickers of "natural" liver diseases. Therefore, hepatic drug reactions range from nonspecific abnormalities of liver tests (which may represent minor degrees of liver injury or hepatic adaptation), through clinicopathologic features of cholestasis, acute hepatitis, and acute liver failure, to more

exotic syndromes such as hepatic sinusoidal or venous outflow obstruction syndromes, nodular regenerative hyperplasia (NRH), chronic hepatitis resembling autoimmune hepatitis (AIH), hepatic fibrosis, NASH, cirrhosis, and benign or malignant liver tumors. It therefore remains crucial that physicians should always consider a possible drug etiology or some other type of hepatotoxicity, irrespective of the pattern of liver injury. A common pitfall is to impute causality to known causes of liver disease that happen to be present (hepatitis C virus [HCV], alcohol, and gallstones are common "confounders") in what are actually cases of drug-induced liver disease. Another challenge is the propensity of some drugs to cause more than one clinicopathologic syndrome of liver injury; examples such as oral contraceptive steroids (OCSs), diclofenac, and nitrofurantoin are discussed later.

Epidemiology and Risk Factors

Epidemiology

In discussing how commonly a drug causes liver injury, it is important to note that the term "incidence" (the number of new cases in a period of time) is not particularly helpful because the onset of adverse drug reactions is nonlinear with time; they tend to occur within the first few weeks or months of treatment. A better descriptor is the proportion of persons exposed to the agent who develop the reaction. This proportion is best described by the *frequency of the reaction* within the affected group; the latter is expressed ideally as the number of persons exposed, but surrogate estimates are often used, such as the number of prescriptions written or the number of person-years of drug ingestion. Other estimates of the frequency of adverse drug reactions or risk of hepatotoxicity come from prescription event monitoring or record linkage conducted prospectively by health maintenance groups and from case–control studies. More commonly used methods include voluntary (or mandated) reporting of reactions to agencies that monitor adverse drug reactions or drug manufacturers. However, this approach is weakened by the inherent inaccuracies of case definition and the vagaries of case documentation, factors that depend on the skill and motivation of observers.

Drugs that carry a high frequency of liver injury are usually recognized as hepatotoxic during phase III trials, which typically involve hundreds or a few thousand persons, or during the first 2 years of postmarketing surveillance (which may involve hundreds of thousands or millions of subjects) (3). Fialuridine, bromfenac, and troglitazone are recent examples of agents withdrawn because of a high frequency of severe liver injury. More often, the recognition of drug-induced liver disease comes several years after the release of a new agent, often with a flurry of case reports, small series, and analyses of larger repositories of information held by drug-monitoring authorities or pharmaceutical companies. More recent examples include amoxicillin–clavulanate, oxypenicillins, diclofenac, sulindac, and troglitazone (21,22,23). Such "miniepidemics" serve principally to highlight how often early reactions to the implicated agents may have been overlooked, or they evolve from massive prescribing of the vigorously marketed new drugs; the latter phenomenon is illustrated by hepatic reactions to flucloxacillin in Australia, where the number of prescriptions written per million people greatly exceeded that of any other country (24).

Reliable information about the risk of liver injury is available for less than 20 of the 300 or so currently used drugs that have been implicated as possible or likely causes of drug-induced liver disease. Some of these data are summarized in Table 33.2. It is reiterated here that a significant issue confounding the epidemiology of this type of disorder is the lack of diagnostic accuracy in defining cases of drug-induced liver disease. As discussed in the next section, this depends entirely on probabilistic evidence surrounding the onset of the

reaction and its resolution or recurrence in relation to drug exposure, the elimination of other known causes of similar hepatobiliary disease, and occasionally on ancillary evidence of an adverse drug reaction.

When drugs contribute to but are not the unique cause of liver disease, it may be possible to assign a relative risk for this complication. This has been attempted in the case of OCS and liver tumors (25,26), OCS and hepatic venous outflow obstruction (27) (see Chapter 40), aspirin and Reye's syndrome (28), and methotrexate and hepatic fibrosis among those who drink appreciable quantities of alcohol (29). Future attempts at providing similar semiquantitation of the risks of drug-induced hepatotoxicity within "high-risk" scenarios should be directed at the use of antiretroviral agents for HIV/AIDS, and tuberculosis or cancer chemotherapy among persons with chronic hepatitis B or C infection.

Risk Factors for Incidence and Severity

The factors that increase the risk of drug-induced liver injury may include dose, duration of treatment, blood levels, age, gender, coincidental metabolic disorders or genetic predisposition to hypersensitivity reactions, concomitant exposure to other drugs or environmental agents, and underlying liver disease. Some examples are summarized in Table 33.4, while further details are given in later sections concerned with individual drugs. A recurring theme is the relationship between failure to recognize a given drug as the possible cause of the patient's symptoms, the resultant failure to discontinue exposure to that drug, and subsequent development of severe hepatotoxicity, often with liver failure and lethal consequences.

Table 33.4. Risk Factors for the Incidence and Severity of Drug-Induced Liver Diseases

Risk factor	Representative agents	Importance

Age	Isoniazid, nitrofurantoin, halothane, troglitazone	Age >60 y increases frequency and severity
	Valproic acid, salicylates	More common in children
Gender	Halothane, minocycline, nitrofurantoin, dextropropoxyphene	More common in women, especially chronic hepatitis
	Amoxicillin–clavulanate, azathioprine	More common in men
Dose	Acetaminophen; some herbal medicines	Risk of hepatotoxicity depends on blood levels
	Anticancer drugs; perhexiline, tacrine, oxypenicillins, dantrolene	Partial relationship to dose
	Methotrexate, vitamin A	Total dose, dose frequency, and duration of exposure influence risk of hepatic fibrosis
Genetic factors	Halothane, phenytoin, sulfonamides	Multiple cases in families, in vitro test results
	Amoxicillin–clavulanate	Strong human leukocyte antigen association
	Valproic acid	Familial cases, association with mitochondrial enzyme deficiencies
Other drug reactions	Isoflurane, halothane, enflurane	Cross-sensitivity reported between these classes of drugs
	Erythromycin, other macrolide antibiotics Diclofenac, ibuprofen Sulfonamides, cyclo-oxygenase 2 inhibitors	
Concomitant drugs	Acetaminophen	Isoniazid, zidovudine, and phenytoin lower hepatotoxic dose threshold and increase severity
	Valproic acid	Other anticonvulsants increase risk
Excessive alcohol use	Acetaminophen hepatotoxicity	Lowers dose threshold, worsens outcome
	Isoniazid, methotrexate	Increases risk of liver injury, hepatic fibrosis
Nutritional status		

Obesity	Halothane, tamoxifen, methotrexate	Increases risk of liver injury, nonalcoholic steatohepatitis, or hepatic fibrosis
Fasting	Acetaminophen	Increases risk of hepatotoxicity
Liver disease	Hycanthone, pemoline Antituberculosis chemotherapy, ibuprofen	Increases risk of liver injury Increases risk of liver injury with chronic hepatitis B and C
Other diseases		
Diabetes mellitus	Methotrexate	Increases risk of hepatic fibrosis
Human immunodeficiency virus/acquired immunodeficiency syndrome	Sulfonamides (cotrimoxazole)	Increases risk of hypersensitivity
Renal failure	Tetracycline, methotrexate	Increases risk of liver injury, hepatic fibrosis
Organ transplantation	Azathioprine, thioguanine, busulfan	Increases risk of vascular toxicity

Genetic factors

It seems likely that host predisposition to idiosyncratic hepatic drug reactions is genetically determined (10,11,12,13). The relatively small number of instances in which familial clustering has been documented may reflect the dual requirements for altered expression of relevant genes and for exposure to particular drugs; halothane, OCS cholestasis, valproic acid (VPA), and phenytoin reactions are those for which more than one case has occured in the same family (14). Phenytoin is an example of the reactive metabolite syndrome (RMS) pattern of severe skin reactions often associated with systemic involvement, among which drug-induced liver disease is common (30). Some of the causative agents, risk factors, and clinical features are summarized in Table 33.5. Individuals have a 25% likelihood of developing adverse drug toxicity if a first-degree relative has experienced a similar reaction; the chances are even higher with other risk factors such as HIV/AIDS, systemic lupus erythematosus (SLE), and antecedent intake of VPA or corticosteroids (Table 33.5).

In addition to determining the expression and inducibility of CYP pathways of drug oxidation, conjugation reactions, and antioxidant enzymes (see Chapter 34), genetic factors encode ATP-dependent pathways of drug elimination from hepatocytes, through the canalicular membrane into bile or through the basolateral membrane into sinusoidal blood (31,32). Regulation of the immune response (presumably including hepatic innate immunity) is also genetically determined, and other critical genes encode the structure of the cytoskeleton, heat shock proteins, and cellular resistance against activated cell death pathways. These are all variables in the pathogenesis of drug-induced liver injury (see Chapter 34). Characterization of the genes that are involved in hepatic reactions to clinically relevant agents provides an outstanding challenge in the field of drug-induced liver disease, as reviewed elsewhere (5). The likelihood that more than one "abnormality" (e.g., genetic polymorphisms, extreme variation) is required before tissue-destructive responses occur would explain the rarity of most reactions. It is already known that inherited defects of mitochondrial metabolism clearly predispose to valproate hepatotoxicity (33) (See "Valproic acid (sodium valproate)"), and there are strong associations between human leukocyte antigens (HLAs) and cholestatic drug reactions to amoxicillin–clavulanate and tiopronin (34,35,36). Weaker associations between HLA molecules and particular types of drug hepatitis have also been reported (37).

Age and sex

The frequency and severity of hepatic drug reactions both increase with age (Table 33.4). The explanations are likely to be multifactorial and include increased exposure; higher probability of multiple drug therapy; biologic effects of aging on drug disposition, especially altered hepatic uptake as the result of decreases in blood flow; and/or diffusion of drugs across the hepatic microvasculature into hepatocytes (38). Some of the many examples of drug reactions that are more common in older people are listed in Table 33.4. Conversely, a small number of drug-induced liver diseases

are more common in children, particularly those that involve mitochondrial injury, such as VPA hepatotoxicity and Reye's syndrome.

Table 33.5. The Reactive Metabolite Syndrome and Hepatic Drug Reactions

Drugs implicated	Risk factors	Clinical and laboratory features
• Sulfonamides • Clozapine • Anticonvulsants (e.g., phenytoin, lamotrigine, phenobarbital, carbamaze pine) • Some nonsteroidal anti-inflammatory drugs • Aminopenicillins • Chinese herbal medicines • Quinolones • Protease inhibitors (e.g., nevirapine, abacavir) • Allopurinol • Minocycline	• First-degree relative with serious rash to same drug (one in four risk), or metabolically cross-reacting drug • Human immunodeficiency virus/acquired immunodeficiency syndrome (100-fold increased risk) • Systemic lupus erythematosus (10-fold increased risk) • Corticosteroids at time of starting drug (4.4-fold increased risk) • Valproic acid at time of starting new anticonvulsant (4- to 10-fold increased risk)	• Onset: 1–6 wk (up to 12 wk) • Sentinel symptoms: Fever, pharyngitis, malaise, headache, periorbital edema, otalgia/ headache, mouth ulcers, rhinorrhea • Serious rash: Erythematous, Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme • Lymphadenopathy (16%), splenomegaly • Hepatic reactions: Cholestasis, hepatitis, granulomas (13%) • Nephritis (9%) • Pneumonitis (6%) • Hematologic (neutropenia, thrombocytopenia) (5%) • Encephalitis/meningitis (5%) • Myositis (4%) • Colitis (2%) • Arthritis, transient hypothyroidism • Blood tests: Neutrophilia (shift to left); atypical lymphocytes, acute-phase reactants (early); eosinophilia (often late)

Women are more likely than men to develop drug-induced hepatitis after exposure to nitrofurantoin, sulfonamides, diclofenac, minocycline, troglitazone, and halothane. Chronic hepatitis caused by the first four of these agents (and historically with methyldopa and oxyphenisatin) has an even higher (80% to 90%) female predominance. Some cholestatic reactions are more common in men (Table 33.4); these include amoxicillin–clavulanate- and azathioprine-induced vascular injury in transplant recipients. The reasons for sex differences in some hepatic drug reactions remain unclear.

Exposure to other drugs and toxins

Patients taking more than one agent have an increased risk of adverse drug reactions, including drug-induced liver disease (14,39,40,41,42,43). Particularly relevant examples include acetaminophen, INH, VPA, and anticancer drugs (44). A possible relationship between agents that alter canalicular bile pathways has also been indicated, including interactive hepatotoxicity between OCS and other drugs to produce prolonged cholestatic reactions (45).

Chronic excessive intake of ethanol is a risk factor for hepatotoxicity with acetaminophen, INH, nicotinamide, and methotrexate.

Nutritional status

Fasting predisposes to acetaminophen hepatotoxicity because of its effects on drug conjugation and oxidation pathways, as well as on hepatic glutathione (GSH) levels. It has also been proposed that malnutrition increases the risk and severity of hepatotoxicity from drugs used to treat tuberculosis (46), but controlled studies are lacking. Overnutrition (obesity) increases the risk of halothane hepatitis. The increased risk of NASH and hepatic fibrosis among those taking methotrexate, estrogens, tamoxifen, or corticosteroids (Table 33.4) is discussed later.

Past history and other medical disorders

Instances of cross-reactivity to similar agents are reported with haloalkane anesthetics (e.g., halothane, enflurane, isoflurane), INH and pyrazinamide, sulfonamides and some COX-2 inhibitors, some NSAIDs, and macrolide antibiotics. Such cross-reactivity is surprisingly uncommon, but a history of any previous adverse drug reaction increases the risk of drug-induced liver injury to other agents. It is again emphasized that *a previous reaction to the same drug* is the single most important factor predisposing to unusual severity of

drug-induced hepatitis (e.g., acute liver failure, chronic liver disease).

Renal failure predisposes to methotrexate-induced hepatic fibrosis and tetracycline-induced fatty liver, while renal and other solid organ transplantation is a risk factor for hepatic vascular injury with azathioprine. Likewise, disorders associated with hepatic venous outflow obstruction, such as veno-occlusive disease (VOD) (now termed the *sinusoidal obstruction syndrome* [SOS]; the terms *VOD* and *SOS* are used interchangeably in this chapter), are attributed to cancer chemotherapeutic agents prescribed during bone marrow transplantation (as well as with radiotherapy) (47). Rheumatoid arthritis, and possibly SLE, appears to increase the risk of salicylate hepatotoxicity and sulfasalazine-induced hepatitis. The risk of drug reactions (including hepatitis) to both sulfonamides and sulfones is greatly increased among persons with HIV/AIDS and also in SLE (Table 33.5), while diabetes (as well as obesity, alcohol, renal failure, and preexisting liver disease—see subsequent text) predisposes individuals to hepatic fibrosis during methotrexate therapy.

Preexisting liver disease

Early studies of chlorpromazine, halothane, and methyldopa reactions clearly demonstrated that other liver disorders, including alcoholic cirrhosis and cholestatic liver diseases, did not predispose to these archetypical examples of idiosyncratic drug hepatitis. On the other hand, for a few agents in which partial dose dependency or metabolic mechanisms appear likely, preexisting liver disease may be a risk factor for incidence and severity of drug-induced liver disease (Table 33.4). These drugs include nicotinamide (niacin), hycanthone, pemoline, and some anticancer drugs (48,49,50,51). The risk of methotrexate-induced hepatic fibrosis is also increased in the presence of other forms of liver disease (52). More recently, interactions between inflammatory forms of liver disease, particularly chronic viral hepatitis, and accentuation of liver injury have been described, including an apparently heightened risk of reactions to antituberculosis chemotherapy, ibuprofen, flutamide, cyproterone acetate, and highly active anti-retroviral treatment (HAART) (53,54,55,56,57,58,59).

Toward Better Outcomes

Prevention

Given the central role of the liver in drug metabolism and disposition, and the rarity of most types of liver injury with what are otherwise valuable therapeutic agents, it is impossible to completely prevent all cases of drug-induced liver disease. General approaches to

primary prevention include appropriate use of drugs (nonpharmacologic approaches whenever possible, optimal choice of agents based on efficacy and safety, case selection, avoiding polypharmacy where possible, avoiding excessive dosage), restricted availability and blister packaging of over-the-counter medication (See "Acetaminophen") (60), physician and public education about possible drug side effects and about how to recognize and what to do about them, and monitoring for adverse drug reactions. Conveying appropriate recommendations about dose limitations for agents such as acetaminophen, nicotinamide, and complementary and alternative medicines (CAMs) would prevent many instances of liver injury.

Careful adherence to dosage guidelines (or use of blood levels) has virtually abolished methotrexate-induced hepatic fibrosis (See "Methotrexate"), tetracycline-induced mitochondrial injury, and aspirin hepatitis. Avoiding repeated halothane administration within 28 days or in people with suspected previous halothane sensitivity would prevent many cases of this serious form of drug-induced hepatitis.

For select agents with known hepatotoxicity, and particularly when treatment is likely to extend for longer than 2 to 4 weeks, it may be appropriate to first establish that the liver test results are normal before starting treatment and then estimate liver tests (liver function test [LFT]) or conduct other safety monitoring during therapy. However, although such "LFT monitoring" is often suggested as one approach to prevent serious outcomes of drug-induced liver disease (particularly by authors of single case reports and by manufacturers who share liability in litigation cases), there is little evidence to support this as a general policy (61,62). Therefore, the high costs and inconvenience of such screening, the need to determine appropriate testing intervals (4 weeks is usually too long for agents that can cause acute liver failure), the weak specificity of abnormal results for identifying serious hepatotoxic potential, and the difficulty of defining a threshold at which the drug should be discontinued all thwart the logistics of monitoring drug treatment with liver tests. It particularly needs to be appreciated that 7.5% of subjects receiving placebo in clinical trials have persistently raised ALT levels (63). In the absence of symptoms, it is difficult to specify a level of ALT abnormality at which treatment should be discontinued. It is generally recommended that the drug should be stopped if ALT exceeds five times the upper limit of normal (ULN) (approximately 250 U/L), but any abnormality of serum bilirubin or albumin concentration or prothrombin time and the presence of any symptoms are clear indications to stop therapy.

In practice, there are few agents for which liver test monitoring is strongly endorsed (see also later sections); these include methotrexate, INH, etretinate and other synthetic retinoids, ketoconazole, anticancer drugs, and prolonged therapy with minocycline. Conversely, the "statins" (β-hydroxy-β-methylglutaryl-coenzyme A reductase inhibitors) are a group of commonly used drugs that rarely cause significant liver injury (62,64), and for which the recommendation (by the manufacturers) for LFT monitoring is now being seriously questioned.

Management

The most important aspect is early recognition and discontinuation of the putative causative agent. At each visit, patients should be warned to report any untoward new symptoms, and particularly fever, systemic symptoms (malaise—"I don't feel these tablets agree with me, doctor"; see also the sentinel symptoms listed in Table 33.5), anorexia, nausea, and vomiting. If patients present after jaundice has developed, it is often too late to avoid a severe reaction because of the potential for developing liver failure or prolonged cholestasis. When patients report symptoms of a possible drug reaction, physicians should immediately check liver test results to establish whether there has been a change from baseline; in cases of doubt, the agent should be discontinued.

After drug discontinuation, most adverse drug reactions will resolve spontaneously, rapidly, and completely, but this is not always the case. Drugs with a prolonged half-life are particularly associated with protracted hepatic drug reactions. Amiodarone, etretinate, ketoconazole, and hypervitaminosis A are examples, but delayed resolution of liver injury can occur with several other drugs on some occasions. In case of severe reactions, hospitalization is advisable, and further evaluation is carried out if the diagnosis is unclear. Otherwise, relief of symptoms is all that is required. As for any type of hepatic injury, older age carries an increased risk of severe liver injury. Repeated vomiting, deepening jaundice, and development of even subtle laboratory or clinical features of liver failure are indications for admission. Transfer to a liver failure unit should be considered and/or discussions with a liver transplant team should be initiated before the patients decline into hepatic coma, or have bleeding from coagulation disorder, sepsis, and hepatorenal failure.

In cases of dose-dependent hepatotoxicity, approaches to management include testing for drug levels and monitoring the clinical condition of the poisoned person. Attempts to remove unabsorbed drug by aspiration of stomach contents should be considered for agents such as acetaminophen, metals, and toxic mushrooms; other approaches (administration of charcoal or other resins or osmotic cathartics) are generally unlikely to be effective, although they have been advocated

for poisoning with toxic mushrooms (65). Likewise, approaches to remove residual drug from the body, such as using chelating resins for drugs with an enterohepatic circulation (66) or by hemodialysis, passage of blood through charcoal columns, or forced diuresis are not effective for most hepatotoxins.

Acetaminophen hepatotoxicity is the only drug-induced liver disorder for which a specific antidote (*N*-acetylcysteine [NAC]) is available (67). Two agents that have been proposed to control protracted hepatic drug reactions are corticosteroids and ursodeoxycholic acid. There are few clear guidelines for their use, and the evidence of efficacy is confined to uncontrolled reports among individual cases or in small series. Older studies with corticosteroids found limited or no efficacy among cases of severe drug hepatitis for methyldopa, iproniazid, INH, chlorpromazine, halothane, phenytoin, and oxyphenisatin (14). More recent observations indicate occasional responses, particularly when drug-induced liver injury is associated with vasculitis (e.g., allopurinol, sulfonamides) and in some (but not all) cases of drug-induced chronic hepatitis (14). A pragmatic approach is to observe the course for 3 to 6 weeks after stopping the drug (unless there is evidence of further deterioration), reserving corticosteroids for cases in which there is failure to show clinical or biochemical improvement or in which the differential diagnosis between AIH and drug-induced chronic hepatitis remains in doubt.

Some experienced clinicians still favor a short course of corticosteroids ("steroid whitewash") to hasten recovery in persons with prolonged drug-induced cholestasis. If the mechanistic basis for such efficacy can be established (stimulating reexpression of the canalicular transporters responsible for bile flow would be a possible example), a firmer conceptual basis or more appropriate pharmacologic agents to stimulate a clinical resolution may become possible. Meanwhile, corticosteroids have a range of unpleasant or severe side effects, and our preference would be to use ursodeoxycholic acid (15 mg/kg body weight) in such cases. There is a reasonable body of uncontrolled data to indicate that approximately two thirds of such cases will respond with reduction of pruritus and other symptoms and acceleration of biochemical improvement. Ursodeoxycholic acid is safe, well tolerated, and has been used with occasional success in patients presenting with cholestatic liver injury attributed to amoxicillin–clavulanate, flucloxacillin, and flutamide (68,69,70,71), as well as cyclosporine (which is not a form of cholestasis) (72). Other approaches to treating pruritus are discussed (73) in Chapter 24. During prolonged cholestatic reactions, fat-soluble vitamin deficiency should be corrected.

Diagnosis of Drug-Induced Liver Disease

Diagnosis of drug-induced liver disease is always presumptive because it is based on a logistic approach rather than on absolute criteria and specific diagnostic tests. As a result, there will be varying degrees of certainty about the diagnosis, depending on the strength of supporting evidence. In estimating the likelihood of diagnosis ("causality assessment") (74,75), evidence is sought by first determining whether the link between drug ingestion and liver injury is plausible, then by excluding other disorders and seeking the presence of any positive features of adverse drug reactions, and finally by assessing features indicative of the liver histology. Attempts have been made to compile these lines of evidence into diagnostic "scales" that give weight to various features (76,77). Regrettably, these fall down in relation to less common or atypical types of drug-induced liver disease, which are those most difficult to diagnose. They have particular limitations for cases with a long delay between the start of drug ingestion and recognition of liver injury (74,77).

Clinical Suspicion

Some situations in which drug-induced liver disease may be particularly likely are summarized in Table 33.6. In such cases, meticulous attention should be paid to the drug history, returning to it as a special investigation, with consideration of nonprescribed medications, CAM, and environmental toxins. It may be pertinent to direct inquiry to other members of the household and primary care providers and to examine all medications being taken or even the contents of drug cupboards, bedside tables and lockers, and so on.

Time of Onset in Relation to Drug Ingestion

Dose-dependent hepatotoxins usually produce overt evidence of liver injury within hours or a few days (See "Acetaminophen"). For adverse hepatic drug reactions, there is a *latent period* between commencing the drug and the development of symptoms and/or abnormal liver test results. With immunoallergic types of drug hepatitis, granulomatous hepatitis, and drug-induced cholestasis, this is within 4 months (most typically 2 to 10 weeks) in more than three quarters of cases. Occasionally, liver injury becomes evident only after the drug is stopped; for amoxicillin–clavulanate this may be up to 6 weeks after discontinuation. With other types of drug hepatitis (presumed to be instances of metabolic idiosyncrasy), the latent period to onset tends to be a little longer, often between 6 and 26 weeks, and with drug-induced chronic liver disease (e.g., chronic hepatitis, steatohepatitis, and syndromes related to

vascular injury), drug exposure may be for 6 months or longer than 1 year before clinical onset is apparent.

Table 33.6. Some Situations in which Drugs are a Particularly Likely Cause of Liver Disease

- The person has started new treatment (including CAM) in last 3 to 6 m
- Presence of extrahepatic manifestations, especially rash, lymphadenopathy, eosinophilia (see also Table 33.5)
- Acute hepatitis not readily accounted for by hepatitis viruses, other infections, or metabolic or immunologic disorders
- There are atypical features of liver disease—mixed "hepatocellular and cholestatic" reactions, hepatitis with microvesicular steatosis
- Cholestasis with normal bile duct caliber on hepatobiliary imaging
- Cholestasis after common causes have been excluded, particularly in the elderly
- Histologic features suggest drug-induced liver disease (see text) in cases of cholestasis or acute hepatitis, and hepatitis with hepatic granulomas
- Chronic hepatitis without autoantibodies or hyperglobulinemia
- Abnormal liver tests in complex medical situations
- Obscure or poorly explained liver disease among those taking sex steroids, immunosuppressive agents, or other drugs (including CAM) for years

CAM, complementary and alternative medicine.

Exploring the *chronologic relationships* between drug ingestion and the onset and resolution of liver injury is the most important consideration in the diagnosis of drug-induced liver disease, as discussed elsewhere (74,75,78).

Repeated Drug Ingestion

Some drugs almost never cause liver injury after first exposure, but the risk of hepatotoxicity increases with each subsequent treatment course. Halothane, dacarbazine, and nitrofurantoin are recognized examples. For a much broader range of compounds, a personal history of previous reaction to the drug (*inadvertent rechallenge*) is common among those with severe or prolonged liver injury; INH, NSAIDs, Chinese herbal medicines, germander, and chaparral are typical examples.

To provide stronger evidence of causality, *deliberate rechallenge* has been used in the past, employing a single and smaller than usual dose of the suspected hepatotoxin; it has proved particularly valuable to incriminate agents not previously known to be associated with liver injury and to identify which agent is responsible when the person has been taking more than one potentially hepatotoxic drug. A positive response (connoted by a recurrence of fever or other symptoms and/or a twofold increase of ALT or serum alkaline phosphatase [SAP] levels) (78) strongly implicates the drug in causing liver injury. However, the practical application of rechallenge is greatly limited by safety considerations; it should never be conducted without the fullest consideration by both the persons involved and their family, and should be done with reference to an Institutional Human Ethics Review Board. Particularly, it should be noted that deliberate rechallenge is potentially dangerous and should never be attempted for the types of reactions listed in Table 33.5.

Response to Discontinuation of the Drug

Dechallenge should be followed by improvement in liver test results within days or weeks of stopping the drug; some guidelines have been provided (78). As for time to onset, there are clear exceptions to this generality. Therefore, in cases of liver disease caused by agents such as ketoconazole, troglitazone, coumarol,

etretinate, amiodarone, and minocycline, severe reactions may resolve slowly (months), or incompletely, with further decline of hepatic function (e.g., troglitazone). Although some instances of drug-induced cholestasis can also be prolonged, failure of jaundice to resolve in case of suspected hepatic drug reactions more often indicates that an alternative diagnosis (e.g., malignant biliary obstruction) has been missed.

Clinical Features

Although the clinicopathologic syndrome associated with exposure to a particular drug may be a useful aid to diagnosis, the diversity of reactions to individual drugs is such that absence of the "drug signature" (or "syndromic recognition") test should *not* be used to exonerate a given drug as the cause of liver injury. In most respects, the clinical features of drug hepatitis or drug-induced cholestasis are not dissimilar from those found with other causes of these disorders. However, identifying specific risk factors for hepatotoxicity (e.g., prolonged fasting or chronic excessive alcohol intake by a person regularly taking acetaminophen) or the presence of extrahepatic features of drug hypersensitivity (Table 33.5) may suggest the correct diagnosis.

Exclusion of Other Disorders

It is critical to exclude other liver diseases before attributing liver injury to drugs (Table 33.7). In an earlier work (79), approximately two thirds of reactions reported as drug-induced chronic hepatitis were subsequently ascribed to chronic hepatitis C. Contemporary

serologic, viral, immunologic, and imaging tests have facilitated the early diagnosis of most acute viral, vascular, and metabolic liver disorders. Likewise, the cause of cholestasis, and particularly mechanical obstruction of the biliary tract, is usually easy to identify with modern imaging (see Chapter 8). Approaches to make the correct diagnosis of a drug reaction when the clinical and laboratory features resemble AIH include the course of the disease after discontinuation of the drug and the patient's response to a short course (4 to 6 weeks) of corticosteroids if there is not rapid improvement after stopping the drug. If there is impressive response to the patient's condition with corticosteroids, followed by relapse after reducing the dose, it allows the physician to assume that the case is actually one of AIH.

Table 33.7. Drug-Induced Liver Disease is a Diagnosis of Exclusion: Consider the Following

- Hepatitis viruses: Serology and molecular virology (especially hepatitis C virus ribonucleic acid)
- Other infectious agents: Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, herpes simplex virus, *Coxiella burnetii*
- Autoimmune hepatitis: Antinuclear antibodies, smooth muscle antibodies, liver/kidney microsomal antibodies, immunoglobulin G levels
- Acute biliary obstruction, exclude cholangitis
- Metabolic disorders—Wilson disease, α_1-antitrypsin deficiency, risk factors for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (serum lipids, fasting blood glucose or abnormal glucose tolerance test, insulin, C peptide, family history of diabetes, features of the insulin resistance [metabolic] syndrome)
- Vascular disorders of liver; risk factors, imaging with vascular phase (see Chapter 8)
- Alcohol
- Bacterial infection
- Hepatic metastases
- Systemic malignancy or lymphoma

Liver Biopsy

Liver biopsy plays a special role in excluding other hepatobiliary disorders, but it may also provide positive evidence to corroborate drug-induced liver injury. Biopsy is most strongly indicated when the cause of liver disease remains in doubt; for example, there may be ambiguous evidence of autoimmunity or the pattern of reaction may be very unusual or not previously reported for the drug in question. Details such as whether continued treatment with this medication is highly desirable and whether there is rapid improvement after stopping the drug may influence the decision of performing liver biopsy. As for any indication, the justification for liver biopsy must satisfy the question "Does the likelihood that this procedure will make a difference to the management of the person justify the inconvenience, discomfort, and risks of the procedure?" Informed consent is clearly mandatory, and it may be valuable to document in the medical record (or on a signed consent form) why the biopsy is considered valuable in this particular case.

Table 33.8. Histologic Changes that may Indicate Drug-Induced Liver Disease

- Zonal lesions, including necrosis and/or steatosis
- Microvesicular steatosis (often results from mitochondrial injury)

- Necrotic lesions of disproportionate severity of the clinical picture
- Mixed hepatitis and cholestasis
- Destructive bile duct lesions
- Prominent neutrophils and (in later stages) eosinophils (>25%)
- Granulomas
- Vascularity of hepatic tumors—sinusoidal dilatation, peliosis
- Vascular lesions
- Florid steatohepatitis—resembles alcohol-related steatohepatitis more than typical "primary" nonalcoholic steatohepatitis

There are no histologic features that are pathognomonic for drug-induced liver disease, and indeed some, such as the presence of occasional eosinophils, are often overinterpreted. Nonetheless, some patterns of hepatic lesions may suggest, to an experienced liver pathologist, that a drug or toxin could be implicated (Table 33.8). The reader is referred to the excellent detailed illustrations found in texts such as Hall (80) and Zimmerman (2), as well as earlier editions of this book.

Specific Diagnostic Tests

As reviewed elsewhere (5,9), there are no completely validated specific tests for any type of drug-induced liver disease. Interesting data have been presented about the relative specificity of some drug-induced autoantibodies, including anti-M6 (antimitochondrial antibodies [AMAs]) with iproniazid, anti-LKM-2 (against CYP 2C9) with tienilic acid, anti-CYP 1A2 with dihydralazine, and anti-CYP 2E1 with halothane. A test that detects antibodies against trifluoroacetylated (TFA) proteins has been advocated for halothane and other haloalkane-related hepatotoxicity (see later section). However, all such tests have minimal applicability in clinical practice because of their unavailability and lack of standardization, including agreement on what comprises a significant titer of antibodies.

Identifying One Causative Agent Among Many

Having established with near certainty that one or more drugs are responsible for an individual case of liver disease, an additional challenge may be to identify which among several is the guilty party. In general, the drug started most recently before the onset of liver injury is the one most likely to be responsible; new and nonproprietary medicines should also heighten suspicion. Otherwise, the drug with the most hepatotoxic track record becomes the prime contender. Whenever possible, all therapeutic agents, or all potential hepatotoxins, should be discontinued. If the patient's condition and laboratory test results improve, the drug(s) that seem(s) unlikely to be responsible can be carefully reintroduced.

Clinicopathologic Syndromes of Drug-Induced Liver Disease

The initial "labeling" of cases with apparent drug-induced liver injury has relied heavily on the profile of liver test abnormalities (Table 33.9) and not histopathology, which provides a more definitive classification. It is noted here that the clinical and laboratory features are not always congruent with the liver pathology, and there is much overlap between categories. Therefore, although the histologic changes usually mirror the biochemical abnormalities, certain caveats should be borne in mind. *First*, alteration of liver enzymes is not synonymous with liver injury and can represent hepatocyte adaptation (see subsequent text). *Second*, liver tests may underestimate the severity of liver disease, as with the fairly modest changes in levels of aminotransferases (ATs) that may accompany acute liver failure from drug-induced microvesicular steatosis. Conversely, drugs such as estrogens can sometimes be associated with a major increase in ATs despite bland cholestasis on biopsy, while other agents (e.g., methotrexate, vinyl chloride, arsenic) can cause cirrhosis with minimal or no change in biochemical tests of liver disease. *Third*, the pattern of liver tests is most often mixed or relatively nonspecific, and this occurs with granulomatous hepatitis, steatohepatitis, cholestatic hepatitis, chronic hepatitis, and minor nonspecific patterns of liver injury.

A relatively simple approach to clinicopathologic classification of drug-induced liver disease is outlined in Table 33.10.

Hepatic Adaptation

Many drugs induce abnormal liver test results without causing symptoms or biochemical evidence of *significant* liver disease. Minor elevations in the levels of ALT may be transient. In reality, these may indicate minor degrees of nonprogressive injury to key organelles such as mitochondria or cell membranes, without causing cell death and without recruiting an inflammatory response. The nonprogression (and often resolution) of these changes may result from induction of protective processes, such as antioxidant and antiapoptotic pathways; in this sense, minor forms of chemical liver injury are probably often followed by adaptation of the liver to withstand continuing or more substantial insults. Experimental work has demonstrated the hepatoprotective effects of a small dose of carbon tetrachloride against subsequent massive poisoning and the

phenomenon of ischemic preconditioning that substantially abrogates subsequent ischemic or reperfusion injury; the mechanisms of these adaptive processes are of potential importance in the understanding of drug-induced liver disease.

Other forms of "hepatic adaptation" include drug-induced hyperbilirubinemia, as observed with agents such as rifampicin, flavaspidic acid, and cyclosporine, and "induction of hepatic enzymes." Drug-induced hyperbilirubinemia is best understood as a direct interference with pathways of bilirubin uptake, conjugation, and canalicular excretion into bile. Sustained elevation of levels of enzymes such as γ-glutamyl transpeptidase

(GGTP) and SAP is seen with phenytoin and other anticonvulsants, rifampicin, alcohol, and warfarin. Because such agents induce hepatic CYP enzymes, this phenomenon is often referred to as *microsomal enzyme induction*; the actual relationships between the activity of drug oxidases and the increased plasma levels of these nonmicrosomal enzymes are less clear. Potential explanations are that the indirect effects of increased CYP enzyme activity on bile acid metabolism produce more "detergent" metabolites that liberate these enzymes from the hepatocyte plasma membranes or stimulate increased synthesis of alkaline phosphatase and GGTP; there is some evidence that both these mechanisms may apply. The important clinical point is to clearly recognize the difference between these biochemical changes and cholestasis; for example, serum bile acid levels increase with cholestasis or liver injury but not with hepatic adaptation.

Table 33.9. Definition of Patterns of Liver Injury

Liver injury	Hepatocellular	Cholestatic	Mixed
ALT >2–3 × ULN	ALT >2–3 × ULN and normal SAP	SAP >2 × ULN	ALT >2–3 × ULN and SAP >2 × ULN
or	*or*	*or*	*or*
>2 × ULN conjugated bilirubin *or* elevated aspartate aminotransferase, SAP, and total bilirubin (one of these must be >2 × ULN) levels	ALT/SAP ratio ≥5[a]	ALT/SAP ratio ≤2[a]	ALT/SAP ratio[a] between 2 and 5
Note that these patterns are a relatively poor guide to the histologic nature of liver disease (see text). [a]The ALT and SAP values are expressed as multiples of the upper limit of normal. ALT, alanine aminotransferase; SAP, serum alkaline phosphatase; ULN, upper limit of normal.			

Table 33.10. Clinicopathologic Classification of Drug-Induced Liver Disease

Category	Description	Examples
Hepatic adaptation	No symptoms; raised GGTP and SAP (occasionally ALT) levels	Phenytoin, warfarin, rifampin
	Hyperbilirubinemia	Rifampin
Dose-dependent hepatotoxicity	Very short interval to onset; symptoms of hepatitis; zonal, bridging, and massive necrosis; ALT >5 × ULN, often >2,000 U/L	Acetaminophen, nicotinic acid, amodiaquine
Other cytopathic liver injury;		
Acute steatosis	Microvesicular steatosis, diffuse or zonal; partially dose dependent; severe liver injury; features of mitochondrial toxicity (lactic acidosis, pancreatitis)	Valproic acid, didanosine, highly active anti-retroviral treatment, fialuridine, L-asparaginase, some herbal medicines, ecstasy

Acute hepatitis	Onset within 1–20 wk; sentinel symptoms of hepatitis; focal, bridging, and massive necrosis; ALT >5 × ULN; extrahepatic features of drug allergy in some cases (Table 33.5)	Isoniazid, dantrolene, nitrofurantoin, halothane, sulfonamides, phenytoin, disulfiram, etretinate, ketoconazole, terbinafine, troglitazone
Chronic hepatitis	Duration >3 m; interface hepatitis, bridging necrosis, fibrosis, cirrhosis; clinical/laboratory features of chronic liver disease; autoantibodies in some cases	Nitrofurantoin, etretinate, diclofenac, minocycline, mesalamine
Granulomatous hepatitis	Hepatic granulomas with varying hepatitis and cholestasis; raised ALT, SAP, GGTP levels	Allopurinol, carbamazepine, hydralazine, quinidine, quinine
Steatohepatitis	Onset delayed (6–18 m); steatosis, focal necrosis, Mallory's hyaline, pericellular fibrosis, cirrhosis; chronic liver disease, portal hypertension	Perhexiline, amiodarone, tamoxifen, toremifene; rarely nifedipine, diltiazem
Cholestasis without hepatitis	Cholestasis, no inflammation; SAP >2 × ULN	Oral contraceptives, androgens, cyclosporin A
Cholestatic hepatitis	Cholestasis with inflammation; symptoms of hepatitis; raised ALT and SAP levels	Chlorpromazine, tricyclic antidepressants, erythromycins, amoxicillin/clavulanate, angiotensinogen-converting enzyme inhibitors
Cholestasis with bile duct injury	Bile duct lesions and cholestatic hepatitis; clinical features of cholangitis	Chlorpromazine, flucloxacillin, dextropropoxyphene, carmustine, paraquat
Chronic cholestasis Vanishing bile duct syndrome Sclerosing cholangitis	Cholestasis present >3 m Ductopenia; resembles primary biliary cirrhosis but antimitochondrial antibodies absent Strictures of large bile ducts	Chlorpromazine, flucloxacillin, trimethoprim- sulfamethoxazole (Table 33.14) Intra-arterial floxuridine, intralesional scolicidals
Vascular disorders	Sinusoidal dilatation, peliosis, noncirrhotic portal hypertension, nodular regenerative hyperplasia, sinusoidal obstruction syndrome (veno-occlusive disease)	Anabolic steroids, oral contraceptives, vinyl chloride, Thorotrast (Table 33.14)
Liver tumors	Focal nodular hyperplasia, hepatic adenoma, hepatocellular carcinoma, angiosarcoma	Anabolic steroids, oral contraceptives, vinyl chloride, Thorotrast (Table 33.14)

ALT, alanine aminotransferase; GGTP, γ-glutamyl transpeptidase; SAP, serum alkaline phosphatase; ULN, upper limit of normal.

A morphologic or ultrastructural consequence of microsomal enzyme induction by drugs is the presence of ground glass cytoplasm in hepatocytes, as seen by light microscopy. This results from hypertrophy of the endoplasmic reticulum, which can be confirmed by electron microscopy.

Drug-Induced Acute Hepatitis

Many drugs are associated with acute hepatocellular injury (Table 33.11). There is a latent period between starting treatment and onset of symptoms or liver test

abnormalities; this tends to be shorter (2 to 6 weeks) for agents clearly associated with immunoallergic mechanisms (see Table 33.5) and more variable and longer for those presumed to be due to metabolic idiosyncrasy. Onset is often with prodromal features of fever, malaise, and other "sentinel symptoms" (Table 33.5), followed by rash, lymphadenopathy, or other systemic features of drug hypersensitivity. Clinical features that resemble acute viral hepatitis soon follow, or they may be the presenting symptoms; anorexia, nausea, vomiting, and lassitude are prominent among these. Jaundice is present in severe cases. AT levels are raised (Table 33.9) proportionately more than those of SAP. Serum bilirubin concentration and indices of hepatic synthetic function such as prothrombin time and serum albumin concentration are variably altered depending on the severity and duration of liver injury.

Table 33.11. Drugs Associated with Acute Liver Failure

ANALGESICS/NONSTEROIDAL ANTI-INFLAMMATORY DRUGS Acetaminophen[a] Bromfenac[b] Diclofenac Etodolac Ibuprofen Leflunomide Nimesulide Oxaprozin Piroxicam Tienilic acid	**CARDIOVASCULAR DRUGS** Amiodarone Captopril Ecarazine Enalapril Lisinopril Labetalol **ENDOCRINE DRUGS** Carbimazole Propylthiouracil Troglitazone[b]	**NEUROPSYCHIATRIC DRUGS** Carbamazepine Chlormethiazole Felbamate Lamotrigine Nefazodone Pemoline Phenytoin Tacrine Tetrabamate Tolcapone Topiramate Valproic acid
ANTIMICROBIAL DRUGS Amoxicillin–clavulanate Ciprofloxacin, ofloxacin, trovofloxacin Cotrimoxazole Dapsone Fialuridine[b] Flucloxacillin Highly active antiretroviral treatment Isoniazid, rifampin, pyrazinamide Ketoconazole, itraconazole Lamotrigine Minocycline Sulfonamides (many) Terbinafine Tetracycline (intravenous)	**ENVIRONMENTAL AGENTS** Carbon tetrachloride[a] Mushroom poisoning[a] **GASTROINTESTINAL DRUGS** Ebrotidine Omeprazole Ranitidine **GENERAL ANESTHETICS** Halothane Enflurane Isoflurane	**ONCOTHERAPEUTIC DRUGS** Carboplatin Chlorambucil Cyproterone acetate Flutamide Gemcitabine Imatinib mesylate **MISCELLANEOUS DRUGS, INCLUDING HERBAL MEDICINES AND SELF-ADMINISTERED AGENTS** Allopurinol Chaparral[a] Cocaine[a] Disulfiram Germander Herbal slimming aids Hydrazine sulfate Interferon-α, interferon-β[a] Kava 3,4-Methylenedioxymethamphetamine ("ecstasy") Nicotinic acid[a] Zafirlukast

[a]Dose-dependent hepatotoxicity.
[b]Withdrawn.

The histologic lesions consist mainly of focal hepatic necrosis, with apoptotic (acidophil) bodies and a mixed inflammatory infiltrate. Bridging necrosis is present in severe cases and may lead to chronic hepatitis if the causative agent is not withdrawn.

Zonal necrosis is a typical feature of more severe forms of liver injury caused by drugs and other chemical or plant toxins. *Zone 3 lesions* (centrilobular or perivenular) are seen with acetaminophen and carbon tetrachloride toxicity but can also occur in acute SOS (or VOD). The overrepresentation of zone 3 lesions in

cases of drug-induced liver injury is related to the high metabolic activity of this zone, which generates reactive metabolites. By comparison, isolated *zone 1* (phosphorus or iron poisoning) and *zone 2 lesions* (cocaine toxicity) are extremely rare.

Severe acute hepatitis may culminate in *acute liver failure*; massive or submassive hepatic necrosis is often present. Acetaminophen (See "Acetaminophen") is the leading cause of drug-induced acute liver failure worldwide, but regional differences exist. Other drugs that have been associated with acute liver failure are listed in Table 33.11.

Mitochondrial Injury

Fatty liver (*steatosis*) can be seen in the vicinity of zonal necrotic lesions but may also be the predominant manifestation. Microvesicular steatosis is a severe form of hepatotoxicity that results from mitochondrial injury; decreased numbers of mitochondria are often found. There is often evidence of other organ involvement, particularly pancreatitis, nephrotoxicity, encephalopathy, and metabolic acidosis. The clinical syndrome resembles acute fatty liver of pregnancy (see Chapter 46). Patients present with nausea, vomiting, abdominal pain, and rapidly evolving encephalopathy. Liver biopsy specimens show accumulation of small fat droplets (microvesicular steatosis) in hepatocytes in a zonal or diffuse distribution. Biochemical features include profound hepatocellular dysfunction with hypoglycemia, coagulopathy, hyperammonemia, and lactic acidemia. There may be a rise in serum bilirubin and AT levels, but these are less pronounced than in other forms of acute hepatotoxicity or hepatitis. Historically, this syndrome was associated with the use of tetracycline in pregnant women, Reye's syndrome (see Chapter 46) caused by the use of aspirin in febrile young children with influenza B and some other viral infections, and with sodium valproate in very young children with predisposing factors (see later section). Currently, the most important cause is HAART, and particularly the nucleoside inhibitors, but amphetamine analogs used recreationally, especially ecstasy, and buprenorphine misuse can produce similar lesions (81). The drugs associated with microvesicular steatosis are listed in Table 33.12.

Unlike acute microvesicular steatosis, drug-induced *steatohepatitis* is a type of hepatocellular injury associated with chronic liver disease. Hepatic decompensation can occur, and some drugs associated with steatohepatitis have also been implicated as causing acute liver failure. In general, however, progression to cirrhosis or liver complications is slower. Drugs associated with steatohepatitis are listed in Table 33.13. The histologic features are indistinguishable from those of alcoholic steatohepatitis, with varying degrees of steatosis, focal lobular inflammation with polymorphonuclear cells, hepatocellular necrosis, and Mallory bodies. Excluding ethanol abuse is critical, but the changes also resemble those found in NASH (see Chapter 39). Therefore, some associations between the drugs used for complications of the metabolic syndrome (e.g., diabetes, high arterial blood pressure, cardiac failure) and steatohepatitis may be fortuitous, while other agents (e.g., corticosteroids, estrogens, tamoxifen) may exacerbate NASH because of effects on insulin resistance and lipid turnover. Drug-induced steatohepatitis often causes hepatic fibrosis in the pericentral (perivenular or acinar zone 3) and pericellular distribution; this distribution is typical for all other causes of steatohepatitis. Cirrhosis can develop and eventually lead to hepatic decompensation.

Table 33.12. Drugs Associated with Microvesicular Steatosis

Aspirin (Reye's syndrome in febrile children)[a]
Fialuridine[b]
Highly active antiretroviral treatment
Industrial toxins—dimethylformamide, selenium
Nonsteroidal anti-inflammatory drugs—ibuprofen, piroxicam, pirprofen, tolmetin[b]
Riluzole
Tetracycline (historical)
Tetrabamate (Atrium)
Ticlopidine
Valproic acid
Vitamin A

[a]Declining incidence since health warnings have been issued on the use of aspirin in young children.
[b]Withdrawn.

Table 33.13. Drugs Associated with Steatohepatitis

Amiodarone
Coralgil (4,4-diethylaminoethoxyhexestrol)[a]
Estrogens[b]

Glucocorticosteroids[b]	
Perhexiline maleate	
Methotrexate	
Methyldopa[b]	
Nifedipine[b]	
Tamoxifen	
Toremifene	
Verapamil[b]	

[a]Withdrawn.
[b]More likely to be a fortuitous association resulting from association with risk factors for "primary" nonalcoholic steatohepatitis (see text).

Cholestasis

There are several different syndromes of drug-induced cholestasis (Table 33.14) (82,83). *Bland cholestasis* occurs without symptoms of hepatitis. Liver biopsies show intrahepatic cholestasis without significant hepatic inflammation. This syndrome is associated with oral contraceptive use and occurs more often in women with a family or personal history of cholestasis of pregnancy (see Chapter 46). Pruritus is often troublesome, but jaundice is less common. Recovery is the rule, but complete resolution can be protracted in some cases.

In *cholestatic hepatitis*, liver histology shows lobular and portal tract inflammation, as well as cholestasis. As with other forms of drug hepatitis, clinical onset is with flu-like symptoms, but this is soon followed by features

of hepatitis, such as anorexia, vomiting, jaundice, and right hypochondrial pain. The latter can be severe and misinterpreted as acute cholecystitis. Drugs that cause cholestatic hepatitis can also cause *bile duct injury*; dextropropoxyphene (84) and methylenedianiline (an epoxy resin hardener associated with cases of liver injury or jaundice after industrial exposure or food contamination, the latter called most infamously as *the Epping jaundice*) cause a striking syndrome of cholangitis (85). Cholangiolytic (interlobular bile duct) injury is prominent in many cases of drug-induced cholestatic hepatitis, and when lesions are severe enough this can lead to ductopenia or the *vanishing bile duct syndrome* (VBDS).

Table 33.14. Clinicopathological Syndromes of Drug-Induced Cholestasis

Type	Clinical and laboratory features	Examples
ACUTE FORMS		
Cholestasis without hepatitis ("bland" cholestasis)	Prodromal symptoms, then intense pruritus; SAP >3 × ULN; AT rise is often transient (rarely >3–5 × ULN); bilirubin <12 mg/dL	Estrogens, anabolic steroids, tamoxifen, azathioprine
Cholestasis with hepatitis[a]	Right upper quadrant or generalized abdominal pain, jaundice often present; can simulate acute cholangitis; pruritus less impressive; SAP >3 × ULN in 70%, AT >2–5 × ULN	Chlorpromazine, other phenothiazines, macrolide antibiotics, oxypenicillins, tricyclic antidepressants, amoxicillin-clavulanate, ketoconazole, nonsteroidal anti-inflammatory drugs (sulindac, piroxicam), captopril, enalapril, azathioprine
Cholestasis with bile duct injury[a] (cholangiolytic)	Resembles acute cholangitis (often with cholestatic hepatitis)	Dextropropoxyphene, flucloxacillin, paraquat, methylenedianiline
CHRONIC FORMS		

Vanishing bile duct syndrome (Table 33.15)	Chronic cholestasis, resembles primary biliary cirrhosis but antimitochondrial antibodies absent	Chlorpromazine, flucloxacillin, and other oxypenicillins
Large bile duct strictures (similar to sclerosing cholangitis)	Chronic cholestasis, resembles sclerosing cholangitis (but strictures at junction of right and left hepatic ducts)	Floxuridine, intralesional scolicides (2% formaldehyde, hypertonic saline, iodine solution, absolute alcohol, silver nitrate)

[a]Several drugs implicated in causing cholestatic hepatitis may also produce bile duct injury.
AT, aminotransferase; SAP, serum alkaline phosphatase; ULN, upper limit of normal.

Table 33.15. Drugs Associated with the Vanishing Bile Duct Syndrome

Amoxicillin–clavulanic acid[a]	Flucloxacillin[a], dicloxacillin
Ampicillin	Glycyrrhizin (a component of Chinese herbal medicines)
Amitriptyline, imipramine	Gold
Azathioprine	Haloperidol
Barbiturates	Ibuprofen
Carbamazepine[a]	Itraconazole
Chlorothiazide	D-Penicillamine
Cotrimoxazole[a]	Phenytoin[a]
Clindamycin	Prochloperazine[a]
Chlorpromazine[a]	Tetracycline[a]
Cimetidine	Terbinafine
Cyproheptadine	Thiabendazole
Erythromycin esters[a]	Tiopronin
Estradiol, norandrostenolone, methyltestosterone[a]	Tolbutamide

Original references can be obtained from refs 2,14,67,83,86.
[a]More than one reported case.

The VBDS is characterized by progressive destruction of segments of the biliary tree (82). Over 30 drugs have been implicated (82,86,87,88,89,90,91) but those with highest risk of this outcome after drug-induced cholestatic hepatitis are chlorpromazine (7% of cases), oxypenicillins (particularly flucloxacillin [10% to 30% of cases]), and erythromycins (5% of cases) (92) (Table 33.15). The main predictor of VBDS after cholestatic hepatitis may be the initial severity of bile duct damage (87). The clinical features resemble those of primary biliary cirrhosis (PBC) with jaundice, with liver test results indicating cholestasis, hypercholesterolemia, xanthomas, and other manifestations of chronic cholestasis. However, unlike PBC, AMAs are usually absent. Hepatic fibrosis is not usually prominent, but biliary cirrhosis can sometimes develop. Resolution may take up to 2 years and is sometimes incomplete.

Large duct bile duct lesions ("sclerosing cholangitis") are an uncommon type of drug-induced injury. Well-characterized historical examples include intra-arterial floxuridine infusions (which cause an ischemic cholangiopathy) and intracavitary instillation of scolicidal agents (82,93). Current practices of targeted chemotherapy for hepatic malignancy and ultrasound-guided scolicidal therapy have a low risk of drug-induced biliary injury.

Granulomatous Hepatitis

Hepatic granulomas are associated with numerous infective, inflammatory, vasculitic, and neoplastic disorders (94) (see Chapter 52). Older studies found that approximately one third of cases of hepatic granulomas were attributable to drugs (95), and it seems likely that drugs remain an important cause of "granulomatous hepatitis." In drug reactions, granulomatous hepatitis may be the sole manifestation of liver injury, but more commonly granulomas accompany other histologic features and represent one of several patterns of hepatic response to injury. Therefore, granulomas have been described with many drugs that are better known for other types of pathology, especially acute hepatitis (e.g., nitrofurantoin, methyldopa, halothane), but also cholestatic hepatitis (chlorpromazine, phenylbutazone, carbamazepine) and steatohepatitis (e.g., amiodarone, calcium channel blockers) (Table 33.16).

The clinical presentation is indistinguishable from other causes or idiopathic granulomatous hepatitis (see Chapter 52). Therefore, profound lethargy, fever, night sweats, rigors, myalgia, and weight loss are prominent. There is tender hepatomegaly and the spleen is enlarged in 25% of cases. Other extrahepatic manifestations include rashes, particularly a form typifying small vessel vasculitis, lymphadenopathy, and bone marrow granulomas (14). Serum ALT level is often raised, but typically less than that of SAP and GGTP. Jaundice is much less common than that with other types of drug-induced acute hepatitis but has been observed when cases overlap with cholestatic hepatitis; carbamazepine and hydralazine are examples. Withdrawal of the offending drug leads to rapid resolution in most cases. A short course of corticosteroids may hasten recovery in special circumstances, particularly in cases associated with vasculitis or other extrahepatic complications such as interstitial nephritis; allopurinol and sulfonamides are examples.

Drug-Induced Chronic Hepatitis

Chronic hepatitis is defined by persistence of symptoms and biochemical or histologic abnormalities for longer than 3 months (14). All drugs implicated as causing chronic hepatitis have also been associated with acute reactions. Continued ingestion of the drug beyond the phase of acute hepatitis is one of the key factors leading to the development of chronic hepatitis, cirrhosis, and liver failure. Early recognition and timely withdrawal of the offending drug are therefore crucial.

A distinctive type of drug-induced chronic hepatitis resembling AIH is described. This probably occurs through the process of drug-induced immune dysregulation. The relatively few agents that cause this type of liver disease include nitrofurantoin, diclofenac, methyldopa, and minocycline. More than 80% of reported cases occur in women. Characteristic features include fever and arthralgia in addition to symptoms of chronic hepatitis and signs of chronic liver disease, hypergammaglobulinemia, and antinuclear and/or smooth muscle antibodies (SMAs). A second pattern of drug-induced chronic hepatitis is characterized by autoantibodies directed against specific hepatic microsomal proteins (e.g., tienilic acid, halothane). However, yet other agents (e.g., etretinate and germander) can be associated with chronic hepatic inflammation and liver cell injury (hepatitis) in the absence of autoantibodies or other features of autoimmunity. Finally, a picture of classical AIH may emerge after an episode of drug-induced acute hepatitis. It is unclear whether the emergence of AIH is related to unmasking of latent AIH or whether the drug may directly induce AIH in susceptible persons.

Table 33.16. Drugs Associated with Hepatic Granulomas

ANTIMICROBIALS	**ANALGESICS/NONSTEROIDAL ANTI-INFLAMMATORY DRUGS**	**ANTIEPILEPTICS**	**MISCELLANEOUS, INCLUDING HERBAL MEDICINES**
Amoxicillin–clavulanate	Aspirin	Carbamazepine[a]	Allopurinol[a]
Cephalexin	Acetaminophen	Chlorpromazine	Chaparral
Dapsone	Clometacin	Phenytoin[a]	Gold
Dicloxacillin, oxacillin	Phenylbutazone[a]	**ENDOCRINE DRUGS**	Glibenclamide
Isoniazid	**CARDIOVASCULAR DRUGS**	Chlorpropamide	Halothane
Mebendazole	Amiodarone	Methimazole	Interferon-α
Nitrofurantoin	Diltiazem	Glibenclamide	Mesalamine
Norfloxacin	Disopyramide	Tolbutamide	Methotrexate
Penicillin G, penicillin V	Hydralazine[a]		Rosiglitazone
Pyrazinamide	Methyldopa		Tacrine
Quinine[a]	Procainamide[a]		Tetrabamate
Sulfonamides (many)[a]	Quinidine[a]		Ticlopidine

[a]Well-characterized examples with causality proved. Note that the high frequency with which hepatic granulomas are found in liver biopsies is such that other associations may be fortuitous.

Hepatic Fibrosis

Hepatic fibrosis may be the end result of chronic hepatitis, chronic hepatotoxicity, steatohepatitis, or chronic cholestasis with bile duct injury. However, some drugs and chemicals such as methotrexate, vitamin A, and arsenic can provoke an intense fibrotic reaction *without* a prominent inflammatory response. With these agents, other profibrogenic risk factors, especially ethanol but possibly also obesity, diabetes, insulin resistance, and iron overload, can accelerate the rate of fibrogenesis (discussed later in relation to vitamin A and methotrexate).

Vascular Lesions

Drugs and chemicals are the most important cause of vascular lesions of the liver. The latter include histologic

curiosities, such as sinusoidal dilatation, to clinically important disorders, such as peliosis hepatis, SOS (VOD), the Budd-Chiari syndrome, NRH, and other causes of noncirrhotic portal hypertension (Table 33.17).

Causative drugs include sex steroids, alkylating chemotherapeutic agents (such as cyclophosphamide, busulfan, melphalan, dactinomycin), and immunosuppressive agents (azathioprine). In current practice, *SOS* (or *VOD*) most often occurs in the setting of bone marrow transplantation, but in the past it was synonymous with pyrrolizidine alkaloid hepatotoxicity resulting from ingestion of brewed herbal tea mixtures ("Jamaican morning sickness") (96) or from plant alkaloid contamination of herbal medicines such as comfrey (97). Epidemics of VOD have also been reported in areas where pyrrolizidine alkaloid–contaminated wheat flour had been consumed, particularly in south Asia and the Middle East. Hepatic vein and portal vein thrombosis can occur in OCS users, although latent or overt myeloproliferative disorders or inherited procoagulants states (such as factor V Leiden) (98) are usually also found in cases of venous thrombosis at these sites (see Chapter 40).

Hepatic Neoplasms

Drugs and toxins are a rare cause of liver tumors (see Chapters 42 and 43), but some associations exist, as exemplified by OCS and *hepatic adenomas*. OCS use is recorded in over 80% of patients with these adenomas, and there is a strong relationship between adenomas and the dose and duration of estrogen therapy (25,99,100). In addition, evidence of estrogen dependence of these tumors is evident; regression occurs in a significant proportion of patients after discontinuing OCS (101). As discussed later, surgery is still required in most cases.

The relationship between estrogens and *focal nodular hyperplasia* (FNH) is more complex and less clear etiologically (See "Oral contraceptive steroids"). As for cavernous hemangiomas, sex steroids, and particularly estrogens, may have trophic effects on these two benign hepatic neoplasms, and particularly on their vascularity, although this remains controversial (102); estrogens cannot, therefore, be regarded as the primary causative factor.

Malignant tumors such as *hepatocellular carcinoma* (HCC) are caused by chronic viral hepatitis in more than 80% of cases (see Chapter 43), but in parts of Africa and Asia ingestion of aflatoxin-contaminated or other fungal toxin–contaminated food may play a synergistic role in carcinogenesis. Estrogens increase the risk of HCC in long-term OCS users, but this effect is weak and is overwhelmed in importance by chronic hepatitis B virus (HBV) and HCV infection in regions endemic for these viruses (26).

Angiosarcoma is an uncommon liver tumor that was classically associated with the radiocontrast agent Thorotrast (see Chapter 43), vinyl chloride monomer, arsenic, and rarely, androgen use.

Table 33.17. Drug-Induced Vascular Disorders and Tumors

	Examples
VASCULAR DISORDERS	
Peliosis hepatis	Anabolic steroids, azathioprine, oral contraceptives, 6-thioguanine, Thorotrast
Hepatic sinusoidal obstruction syndrome (venous outflow obstruction, including veno-occlusive disease)	Oral contraceptives, pyrrolizidine alkaloids, 6-thioguanine, dacarbazine, gemcitabine
Noncirrhotic portal hypertension	Vitamin A, methotrexate, arsenic, vinyl chloride, azathioprine
Nodular regenerative hyperplasia	Azathioprine, 6-thioguanine, busulfan
TUMORS	
Hemangioma	Oral contraceptives (trophic effect on preexisting lesions only)
Focal nodular hyperplasia	?Oral contraceptives (see text)
Hepatic adenoma	Oral contraceptives, anabolic steroids

Hepatocellular carcinoma	Anabolic steroids, danazol, oral contraceptives, Thorotrast, vinyl chloride
Angiosarcoma	Vinyl chloride, thorium dioxide, arsenic
?, recent data refute existing views of a possible association with oral contraceptives.	

Role of Drugs in Multifactorial Liver Disease

Viral Hepatitis

Patients with chronic hepatitis B or C may be at increased risk of liver injury during chemotherapy for tuberculosis, with cancer chemotherapy, and after intake of ibuprofen and possibly other NSAIDs (56,57,58).

A common problem involves the patient who regularly attends the liver clinic with mild-to-moderate ALT level elevation associated with chronic viral hepatitis, who then presents with an ALT level greater than 300 U/L. Particularly for chronic hepatitis C, changes due to hepatotoxic drugs (56) are more likely than spontaneous fluctuations of disease severity; drugs are almost always the cause with ALT values that greatly exceed 1,000 U/L. A common culprit is acetaminophen taken in moderate daily dosage over a period when there are factors such as concomitant medication, prolonged fasting, or chronic alcohol excess (see later section). Another frequent scenario is the patient who has tried Chinese herbal medicines or some other form of CAM.

Highly Active Antiretroviral Treatment

Abnormal liver test results and clinical evidence of liver disease are common in patients with HIV/AIDS. Contributory factors include chronic hepatitis B or C, hepatobiliary infections and infestations, lymphoma and other tumors, and possibly direct effects of HIV infection (see Chapter 51). However, the most common cause of liver injury is hepatotoxicity from drugs used to treat HIV/AIDS. When monotherapy was used against HIV, individual nucleoside analogs were associated with uncommon episodes of severe cytopathic liver injury, as illustrated later for didanosine. In clinical studies, zidovudine and didanosine have been most often implicated in hepatotoxicity, but no particular nucleoside or nucleotide analog appears more hepatotoxic. As discussed later, it seems likely that drug combinations that include protease inhibitors and nucleoside/nucleotide analogs are more toxic than individual agents.

Bone Marrow Transplantation

As discussed in Chapter 60, hepatobiliary complications of hematopoietic cell transplantation are common, are often serious, and may be multifactorial in origin. Bone marrow transplantation particularly increases the risk of vascular complications, such as VOD and NRH. Therefore, although SOS (or VOD) causes complications in at least 1% of cases during the use of anticancer drugs, the risk of developing this complication can be as high as 54% after bone marrow transplantation, depending on the regimen used (103,104,105). Of 103 patients undergoing bone marrow transplantation in one series, NRH was present in 23% and SOS (VOD) in 9% (106).

The clinical features are similar to those of portal hypertension, often complicated by bleeding esophageal varices and ascites. Hepatic encephalopathy can occur after an episode of severe upper gastrointestinal hemorrhage. Liver test results may be normal or show minor nonspecific changes. The diagnosis is made histologically, although a wedge biopsy may be necessary. In general, the prognosis for NRH is good; complete reversibility may occur in some drug-induced cases (106).

Metabolic Syndrome and Nonalcoholic Steatohepatitis

Steatohepatitis is a form of chronic liver disease in which fatty change is associated with focal liver cell injury, Mallory's hyaline, inflammation with mixed cellularity including polymorphonuclear cells, and progressive hepatic fibrosis in a pericentral and pericellular distribution (see Chapter 39). While alcohol is a common etiology, NASH can be associated with diabetes, obesity, and several drugs (e.g., perhexiline maleate [107] and amiodarone [108]).

Among drugs associated with steatohepatitis during the 1990s and the 21st century, causality is harder to prove because NASH is such a common disorder among patients with insulin resistance or metabolic syndrome (see Chapter 39). Therefore, calcium channel blockers used for arterial hypertension have been associated with steatohepatitis (109,110), and methyldopa has been reported to cause cirrhosis in obese middle-aged women (111); these associations may be fortuitous. Other drugs, including estrogens (112), tamoxifen, and glucocorticosteroids, may precipitate NASH because of their effects on the metabolic risk factors: Insulin

resistance, type 2 diabetes, obesity, and hypertriglyceridemia.

Postoperative Jaundice

Drug-induced liver disease is a common cause of postoperative liver disease. The differential diagnosis of cases with jaundice is considered in Chapter 25. It is usually easy to distinguish underlying hepatobiliary disorders and bilirubin transport abnormalities resulting from tissue hypoxia or systemic infection ("benign postoperative cholestasis" or "jaundice in sick patients") resulting from drug reactions. Halothane-induced liver injury is one of the classic types of hepatic drug reactions but has become much less common now that halothane has been almost completely

replaced (in some countries) with equally acceptable and less hepatotoxic agents. Other than halothane, which causes a typical "signature syndrome," few anesthetics cause significant liver injury. Some cases have been attributed to isoflurane; the evidence and practical implications are discussed in a later section. Postoperative liver injury is also observed with antibiotics (especially amoxicillin–clavulanate, less commonly synthetic penicillins or cephalosporin derivatives), analgesics (particularly acetaminophen given alone or in drug combinations), dextropropoxyphene, tranquilizers, antidepressants, and (undisclosed) use of herbal medicines.

Liver Disease Associated with Particular Classes of Drugs

Antimicrobial Agents

Penicillins

Hepatic injury associated with natural penicillins (e.g., penicillin G, penicillin V) is poorly documented (14). It is usually recorded in the setting of anaphylaxis, suggesting a possible ischemic basis for liver injury rather than direct toxicity (113). In contrast, the semisynthetic penicillins have been implicated in many hepatic drug reactions, including hepatocellular injury, bland cholestasis, and cholestatic hepatitis that may lead to VBDS (114,115,116) (Table 33.18).

Oxacillin

There are many reports of abnormal liver test results, mainly raised AT levels (117,118), with oxacillin. Isolated instances of cholestatic hepatitis or acute hepatitis have also been reported. Onset is within 2 to 24 days. Although eosinophilia has been described, features of hypersensitivity are not conspicuous. The liver test abnormalities usually normalize when the drug is withdrawn.

Flucloxacillin

Over 600 cases of cholestatic hepatitis have been reported, mainly from Europe, Scandinavia, and Australia (119,120,121,122,123). The frequency of liver injury is between 1 and 10 cases per 100,000 persons exposed (120,122). Risk factors for flucloxacillin-induced liver injury are high daily doses, prolonged courses (>2 weeks), and age over 55 years (120). Symptoms usually begin after 1 to 9 weeks but can be delayed for up to 6 weeks after the antibiotic course is completed. Nausea, anorexia, and vomiting herald the onset of hepatitis

and are followed by jaundice and pruritus. Constitutional symptoms and weight loss are often striking. Blood tests reflect severe hepatitis with high AT, SAP, and GGTP values. Bilirubin levels can be markedly raised (122). Liver histology shows cholestatic hepatitis with bile duct injury and ductopenia (121). This is a very severe form of drug-induced liver disease, and the reported mortality is up to 5% (20). Although most patients recover after drug withdrawal, prolonged cholestasis is observed in 10% to 30%, and in some cases this can lead to cirrhosis. Management is supportive in the acute phases, but with continued cholestasis ursodeoxycholic acid appears to benefit approximately two thirds of cases (69). The postulation of an idiosyncratic basis for flucloxacillin liver toxicity is supported by the identification of reactive metabolites capable of inducing cytotoxicity in biliary epithelial cells (124).

Table 33.18. Hepatic Injury Associated with Antimicrobial Agents

Drug	Pattern of liver injury	Comments
Penicillin G Penicillin V	Acute hepatitis, granulomatous hepatitis	Very rare; hypersensitivity features often present
Ampicillin	Acute hepatitis, mixed hepatocellular–cholestatic injury, granulomatous hepatitis	Rarely, vanishing bile duct syndrome; cross-hepatotoxicity with cefuroxime
Ampicillin–sulbactam	Cholestatic hepatitis	Single case

Amoxicillin, carbenicillin	Minor, nonspecific increase in aminotransferases	—
Amoxicillin–clavulanate	Cholestatic hepatitis with bile duct injury; acute hepatitis (15% of cases)	Associated with vanishing bile duct syndrome; granuloma; cirrhosis (single case)
Flucloxacillin	Cholestasis with bile duct injury; vanishing bile duct syndrome	Similar toxicity with oxacillin, cloxacillin, and dicloxacillin, but possibly less frequent, less severe
Cephalosporins	Minor liver injury, acute hepatitis, cholestatic hepatitis	Rare; mild, reversible; granulomas (cephalexin); biliary sludge (ceftriaxone)
Chloramphenicol	Hepatocellular, cholestasis	Hepatic injury rare
Cotrimoxazole	Acute hepatitis, cholestatic hepatitis, granulomatous hepatitis, vanishing bile duct syndrome	Cholestasis recorded with trimethoprim alone; increased risk with human immunodeficiency virus/acquired immunodeficiency syndrome
Erythromycin	Cholestatic hepatitis	Also with azithromycin, clarithromycin, roxithromycin
Minocycline	Acute and chronic hepatitis	Autoimmune hepatitis–type features (see text)
Nitrofurantoin	Acute and chronic hepatitis, granulomatous hepatitis, cirrhosis	Declining incidence; was common in long-term users (>6 m)
Quinolones	Mainly cholestasis; rarely hepatitis, fulminant hepatic failure;	Overall low incidence; granulomas (norfloxacin)
Sulfonamides	Acute hepatitis, cholestatic hepatitis, granulomatous hepatitis, vanishing bile duct syndrome	Similar toxicity with sulfones, sulfasalazine, pyrimethamine–sulfadoxine
Tetracycline	Microvesicular steatosis	Rarely, vanishing bile duct syndrome
Trovafloxacin	Fulminant hepatic failure	Now withdrawn

Cloxacillin and dicloxacillin

Some data indicate that cholestatic hepatitis may be less common and possibly less severe with cloxacillin and dicloxacillin, compared with flucloxacillin (123), but this remains unproved and controversial. In other respects, the liver injury and clinical course resemble the flucloxacillin reaction described earlier (122).

Amoxicillin–clavulanate (Augmentin)

At least 150 cases of cholestatic liver injury have been attributed to amoxicillin–clavulanate (34,35,125,126). The clavulanate component has been implicated because such toxicity is rare with amoxicillin, whereas other clavulanate–penicillin compounds such as ticarcillin–clavulanate (Timentin) can cause cholestasis (127,128). The frequency of liver injury has been estimated at 1.1 to 2.7 cases per 100,000 persons exposed (22). Male gender, age over 60 years, and prolonged courses of treatment are risk factors for hepatic injury. Although there is a strong association with DRB1 *1501-DRB5*0101-DQB1*062 (34,35), this haplotype does not appear to influence the clinical expression and outcomes of amoxicillin–clavulanate hepatitis.

Onset is within 6 weeks (mean 18 days) of beginning therapy; rarely, the onset of symptoms may be up to 8 weeks after completion of treatment (129). Hypersensitivity features such as fever, rash, and eosinophilia are seen in 30% to 60% of patients (113). Bland cholestasis or cholestatic hepatitis is observed in biopsied cases (125). Bile duct injury and perivenular bilirubinostasis with ceroid pigment deposits are often present. Other histologic features include hepatic granulomas, biliary ductopenia (34,129,130,131,132), and rarely, cirrhosis (133). Most patients recover but this can take up to 4 months. Fatalities are rare (134).

Quinolones

Transient increases in AT levels are recorded in 2% to 3% of fluoroquinolone recipients. Serious liver injury is infrequent, with the exception of *trovafloxacin*, which was withdrawn because of severe hepatotoxicity (135). Among the reported cases, cholestatic liver injury predominates, usually occurring within 2 to 21 days (82,136,137,138). *Ciprofloxacin* (137), *norfloxacin* (138), *ofloxacin* (139), *gatifloxacin* (140), and *moxifloxacin* (141) have all been implicated. Instances of hepatocellular injury have also been recorded, including acute liver failure with ciprofloxacin (82,142) and ofloxacin (139). Acute cholestatic hepatitis followed by prolonged cholestasis and biliary ductopenia were observed with gatifloxacin (140). Two elderly subjects with renal impairment developed acute hepatocellular injury with *levofloxacin*, presumably a consequence of altered pharmacokinetics because levofloxacin undergoes renal elimination (143).

Tetracycline

Tetracycline hepatotoxicity was characterized by microvesicular steatosis, resulting in acute liver failure. Risk factors included the administration of high intravenous dosages (usually >2 g/day) of tetracycline to women during pregnancy (15,144) or to men while taking estrogens. The other important risk factor was renal failure, which reduces tetracycline clearance. The clinical features resembled those now seen in some cases of severe liver injury associated with HAART (see later section). Tetracycline hepatotoxicity is attributed to impaired hepatic lipid transport and inhibition of mitochondrial β-oxidation of fatty acids (2). It is no longer observed, now that tetracycline is contraindicated in pregnancy. Oral tetracycline has been associated with two cases of prolonged cholestasis with bile duct injury; bilirubin levels remained elevated for approximately 3 years in one of these patients (90).

Minocycline

Minocycline, a semisynthetic tetracycline used in treating acne, is an important cause of drug-induced liver disease (145,146,147). A systematic review identified 65 published cases, while 493 (6%) of the over 8,000 adverse drug reactions attributed to minocycline reported to an international pharmacovigilance center were liver related (148). Two modes of presentation are described. Those presenting with "early onset" hepatitis (within 5 weeks) exhibit prominent hypersensitivity features such as exfoliative dermatitis, lymphadenopathy, and eosinophilia; this is typical of the RMS (Table 33.5). Among such cases, 6 of 16 were in patients of African Caribbean ethnicity, suggesting possible racial susceptibility. By contrast, long-term minocycline

recipients (>12 months) present with a clinical and histologic picture simulating AIH, with fever, arthralgia (>70%), antinuclear antibodies (ANAs) and/or SMAs (>90%), and raised level of globulins (149). Other features of drug-induced SLE may be present, including arthritis and nephritis (145). The absence of tissue eosinophilia in some cases may divert attention from a drug etiology. Approximately half (48%) of those affected have been women, most (94%) younger than 40 years. This reflects, in part, the age of those exposed to prolonged minocycline for the treatment of acne.

Most patients recover completely after minocycline is discontinued. However, both acute and chronic hepatitis can be severe, with a few patients needing liver transplantation or dying from liver failure (150,151). Occasionally, patients seemed to benefit from a short course of immunosuppressive therapy, but it is unclear whether spontaneous resolution would have occurred. Among fatal cases, additional factors such as myocarditis or concurrent viral infections may have contributed to the mortality (148).

It is unclear how minocycline induces AIH. The sera of one affected patient contained antibodies that reacted with 50- and 90-kDa proteins expressed by human HCC cell lines and with rat CYP proteins (152); this raises the possibility of molecular mimicry between drug-induced autoantibodies and host proteins. The suggestion that minocycline could have triggered latent AIH seems less plausible because most affected patients recover without immunosuppression, and hepatitis occurs in those who do not exhibit the usual HLA haplotypes (HLA-DR3 and HLA-DR4) associated with AIH (153).

Sulfonamides

Sulfonamides have long been implicated as causing acute and chronic hepatitis; cholestatic, granulomatous, or mixed reactions; and rarely fulminant hepatic failure (113,154,155). Persons with HIV/AIDS (156) are particularly susceptible, implicating immune dysregulation in the pathogenesis of the hepatic injury. On the other hand, 90% of patients with sulfonamide hypersensitivity in one series were identified as slow acetylators (157,158); reduced activity of the acetylation pathway may contribute to defective detoxification of a sulfonamide metabolite or facilitate the CYP-mediated production of a nitroso metabolite (14,159). How this triggers an apparent immune liver injury is unclear.

Sulfonamide-induced liver injury usually occurs in the setting of systemic drug hypersensitivity (Table 33.5). Symptoms begin early (within 2 weeks of starting therapy). Associated features include a rash (occasionally

with the Stevens-Johnson syndrome), vasculitis, lymphadenopathy, pancreatitis, neuropathy (113), pancytopenia, and renal failure (158).

Sulfonamide combinations: Cotrimoxazole, sulfasalazine, sulfadoxine, and pyrimethamine (Fansidar)

In addition to other types of sulfonamide-induced liver injury, prolonged cholestasis with biliary ductopenia has been attributed to cotrimoxazole (160,161). Significant liver injury has occurred in recipients of sulfasalazine (see subsequent text) and pyrimethamine–sulfadoxine (Fansidar) (162).

Sulfasalazine and mesalamine

Sulfasalazine has been associated with several reports of acute hepatitis (163,164,165,166,167). Sulfasalazine-associated hepatic injury was observed more often in patients with rheumatoid arthritis than in those with inflammatory bowel disease (168). Reactions are often severe, and at least ten deaths have been recorded. Fever, rash, and arthralgia usually develop within 1 to 4 weeks. Liver injury was originally ascribed to the sulfonamide component (sulfapyridine). However, other 5-aminosalicylic acid (5-ASA) compounds, including *mesalamine* (*mesalazine*) and *olsalazine*, can also cause acute hepatitis, implicating the 5-ASA moiety in at least some cases (169,170). Sulfasalazine- and mesalamine-related hepatic drug reactions occur with similar frequency (168). Mesalazine has also been associated with cholestasis (170a) and rarely chronic hepatitis (one report) with hypergammaglobulinemia, ANAs, and SMAs (169).

Dapsone (4,4-diaminodiphenylsulfone)

Indications for dapsone include leprosy, dermatitis herpetiformis, and increasingly, treatment of *Pneumocystis carinii* infection in HIV-positive individuals. Liver involvement as part of a severe hypersensitivity syndrome ("sulfone syndrome") is well described with dapsone. This reaction, another example of the RMS (see preceding text), is reported in up to 1.3% of dapsone recipients (171). Most cases occurred within 6 weeks. Liver histology shows acute hepatitis or cholestatic hepatitis. Hepatic granulomas or acute cholangitis are also described (172). Resolution occurs within 4 weeks of stopping dapsone. Acute liver failure and death are uncommon but can occur in severe cases. Corticosteroids have been used with success in some reports. However, untreated patients have also recovered, while fatalities are recorded despite corticosteroid use (171,172).

Nitrofurantoin

Cases of significant hepatotoxicity still occur with this formerly prescribed urinary tract antiseptic; in a recent survey from Michigan, nitrofurantoin accounted for 3 of 32 (10%) cases of drug-induced hepatitis (173).

Acute and chronic hepatitis are the most characteristic hepatic syndromes, but varying degrees of cholestasis can occur, as do granulomas in rare cases.

Nitrofurantoin-induced chronic hepatitis simulates AIH. It is seen only in individuals using nitrofurantoin for more than 6 months; in some cases, exposure is intermittent and to very small doses of nitrofurantoin, such as those found in cow's milk (174). Positive rechallenge is well documented and may occur even after 17 years (175). The clinical features are those of chronic hepatitis with fatigue, malaise, nausea, abnormal liver test results with raised level of ALT, hypoalbuminemia, and hyperglobulinemia. ANAs (80%) and SMAs (70%) are often present. Cirrhosis is present in up to 20% of patients with nitrofurantoin-induced chronic hepatitis. Nitrofurantoin is also an important cause of pulmonary fibrosis, and concurrent liver and lung injury are observed in approximately 20% of persons developing nitrofurantoin hepatitis (176).

Clinical recovery follows nitrofurantoin withdrawal, but the course may be prolonged and sometimes recovery is incomplete (2). Corticosteroids are not routinely used but patients taking these medications seem to have accelerated recovery in occasional cases (177). Monitoring liver tests is not likely to be clinically useful or cost-effective.

Erythromycins and other macrolide antibiotics

Erythromycin estolate was recognized as a paradigm of cholestatic hepatitis in the early 1970s, but similar toxicity, although far less common, has been reported with all the erythromycin esters (rarely with erythromycin base) and other macrolides, such as clarithromycin (14). The presence of fever, serum, and tissue eosinophilia, along with the accelerated response to rechallenge, is consistent with immunoallergic idiosyncrasy, although intrinsic toxicity may also contribute (178).

Symptoms begin 2 to 25 days after commencing treatment (114). Nausea, anorexia, vomiting, and abdominal pain are common; the abdominal pain may be quite severe and it mimics acute cholecystitis. Before this syndrome was appreciated, inappropriate cholecystectomy was often performed. Dark urine (bilirubinuria), jaundice, and itch may also occur. The biochemical profile shows raised AT and alkaline phosphatase levels, with hyperbilirubinemia in severe cases. Peripheral eosinophilia can occur. Liver biopsies show intrahepatic cholestasis and portal inflammation, often accompanied by numerous eosinophils. Rare cases have been associated with chronic cholestasis, with biliary ductopenia on histologic sections.

Most recover after the drug is withdrawn, although liver test abnormalities may take up to 4 months to subside (87). Cross-sensitivity between erythromycin preparations has been reported (113). Intravenous

erythromycin lactobionate (179) has been implicated in a case of fulminant hepatitis.

Clarithromycin (180,181), *azithromycin* (182,183), and *roxithromycin* (184) have also been associated with cholestasis or mixed hepatocellular–cholestatic injury. Two reports of fulminant hepatic failure have accrued for clarithromycin; one of these patients was also receiving disulfiram (see "Disulfiram") (185,186).

Antituberculous Drugs

Of the current antituberculous drugs, hepatotoxicity is an important complication of INH, rifampin, and pyrazinamide, particularly when used in combination. Risk factors for liver injury are shown in Table 33.19. Except for a single report of cholestasis (187), *ethambutol* appears to be devoid of hepatic adverse effects. Among drugs used in the past, severe hepatocellular injury and cholestasis were recorded with *p-aminosalicylic acid*, often as part of a multisystem syndrome with hypersensitivity features (2); *prothionamide* (hepatocellular injury); and *ethionamide* (2). Ethionamide is still used rarely as a second-line antituberculous drug. Rifabutin causes liver enzyme changes, but these are usually part of hepatic adaptation; hepatitis has not been a problem.

Isoniazid

Frequency and risk factors

The hepatotoxic potential of INH was established by landmark studies in the late 1960s and early 1970s (188,189,190,191). Minor (less than threefold) elevations in the level of serum AT occur in 10% to 20% of subjects during the first 3 months of treatment. However, these AT abnormalities often settle with continued treatment and a progressive rise is uncommon. Jaundice occurs in approximately 1% of recipients of INH but in more than 2% of those aged 50 years or older. Conversely, liver injury is rare in persons younger than 20. Women, especially of African American and Hispanic ethnicity, and

persons with a history of excessive alcohol use are also at increased risk of toxicity (190,192). The presence of hepatitis B, hepatitis C, or HIV has been suggested as a risk factor (54,193). The relative risk of developing hepatotoxicity if the person was hepatitis C or HIV positive was 5-fold and 4-fold, respectively, and 14-fold in case of HIV/HCV coinfection. Antituberculous therapy could be reinstituted after successful antiviral therapy against HCV. In another study, persons who were hepatitis B surface antigen (HBsAg) positive were more likely to sustain liver injury than HBsAg-negative individuals (9). Further, those with positive replication status (i.e., HBeAg positive) had a higher likelihood of developing hepatotoxicity with INH than those with "inactive" hepatitis B (7.7-fold increased risk). Discontinuation rates were also higher in the former group. Opposing views were expressed in a case–control study (194) in which malnutrition was the only determinant of hepatotoxicity.

Table 33.19. Risk Factors for Antituberculosis Treatment–Related Hepatotoxicity

Age over 60 y
Serum albumin <35 g/L
Female gender
Increased serum bilirubin, preexisting chronic liver disease
Hepatitis B surface antigen positivity
Genetic factors: Human leukocyte antigen haplotype associations, glutathione-*S*-transferase mutations
Multidrug regimens, particularly those containing pyrazinamide
Excess alcohol consumption
CYP 2E1 status

Evidence regarding the influence of the acetylator phenotype has been conflicting. Initial studies conveyed the impression that fast acetylators are at increased risk, but other reports have suggested either that the acetylator status is not a predictor of toxicity (195,196) or that slow acetylators are more susceptible (197,198).

Metabolic idiosyncrasy is postulated as the basis for INH toxicity. Therefore, a CYP-generated toxic metabolite of INH induces oxidative stress–mediated injury to mitochondria or other key organelles. This could explain why the risk of severe liver injury is enhanced by concomitant intake of rifampicin (197) and why pyrazinamide (which is structurally similar to both INH and nicotinamide, a dose-dependent hepatotoxin discussed later) is also associated with an increased risk of more severe hepatotoxicity. Persons carrying certain genetic polymorphisms in key enzymes (e.g., CYP 2E1) that enhance reactive metabolite formation or that are involved in detoxification processes (e.g., glutathione-*S*-transferase) (199) may be predisposed to INH-induced liver injury. An association with certain HLA haplotypes (absence of HLA-DQA1*0102 antigen and the presence of HLA-DQB1*0201 antigen) was observed in North Indian patients (200) (Table 33.19).

Clinical features, outcome, and management

Prodromal features are those of nonspecific drug reactions or resemble those of viral hepatitis. Because of the often vague nature of these symptoms, patients taking INH should be warned (repeatedly) about the potential significance of malaise, fatigue, anorexia, nausea, vomiting, and dark urine. Jaundice is the presenting symptom in 10% of patients. Fever, rash, eosinophilia, or other manifestations of drug hypersensitivity are rare. Patients present within 4 weeks of starting INH in 15% of cases; half of them present within 2 months, but the onset may be delayed from 3 to 12 months in the remainder. A marked rise of AT levels is common, with peak values as high as 4,000 IU/L. Liver biopsy shows lobular hepatitis, often with marked hydropic changes of hepatocytes, and bridging (submassive) or massive (panlobular) hepatic necrosis in fatal cases. Cases associated with apparent chronic hepatitis (without autoimmune features) and cirrhosis have been described rarely, mostly before HCV testing was introduced. These seem to be cases of chronic hepatotoxicity not chronic hepatitis, and patients generally recover after discontinuation of the drug.

Rapid resolution of symptoms and liver test abnormalities occurs when INH is stopped before the onset of liver failure. The almost universal feature of cases leading to liver transplantation or fatal liver failure, from the early 1970s until the present time (190,201) is continued ingestion of INH after commencement of symptoms. The case fatality of patients with jaundice exceeds 10%. Management of liver failure is supportive; corticosteroids are of no benefit. Liver transplantation has been performed successfully (201).

Prevention

In the United States, a 9-month course of INH is recommended for tuberculin-positive family members of patients with tuberculosis. Monitoring of ALT, aspartate aminotransferase (AST), and bilirubin level is recommended at baseline in high-risk groups (e.g., HIV-positive individuals, those with a history of alcoholism, individuals with previous liver disease, pregnant women, and those presenting in the postpartum period) and during treatment (if baseline values are abnormal and in high-risk groups) (202). It is now generally agreed that patients taking INH (or other forms of antituberculosis chemotherapy) should be advised to seek medical attention as soon as new symptoms develop, even if these seem nonspecific and relatively minor. Biochemical monitoring does not effectively substitute for clinical monitoring (203). Therefore, one study found that no more than 0.15% of over 11,000 clinically monitored patients receiving INH developed significant hepatotoxicity (202). In contrast, the most noteworthy aspect of US case reports of liver failure attributable to antituberculosis chemotherapy that resulted in death or liver transplantation has been the failure of patients to report early symptoms and/or failure of health care workers to recognize the significance of these symptoms, thereby allowing continued intake of INH (190,201).

Rifampin

A meta-analysis showed that regimens combining INH and rifampin were associated with a higher incidence of hepatotoxicity (2.5%) than did regimens without this

combination (1.1%) (204). Rifampin probably increases both the risk and severity of INH-associated liver disease (197). The potent CYP-inducing properties of rifampin could facilitate the production of reactive INH metabolites.

On some occasions, rifampin may itself be hepatotoxic, as shown by cases with positive rechallenge and in situations where it is used alone (e.g., to relieve pruritus) (205). In the latter setting (typically in persons with PBC), the frequency of rifampin hepatitis (7.3% to 12.5%) may be higher than what is generally recognized (206). Liver test result changes usually occur within 2 months, rarely as late as 14 months; they mostly conform to a mild hepatocellular pattern (207). The clinical features resemble viral hepatitis. Histologic features include focal hepatocellular necrosis and apoptosis (acidophilic bodies), especially in zone 3; portal inflammation; and cholestasis. Patients recover when rifampin is stopped. However, continued use of rifampin has been associated with jaundice and impaired hepatic synthetic function; histology showed severe interface and intra-acinar hepatitis together with bridging and confluent necrosis. Therefore, new recipients of rifampin (monotherapy) should be prescribed in lower dosages (150 mg/day) initially and the dosage titrated to the clinical response. Rifampin can also be associated with hyperbilirubinemia, possibly because of impairment of canalicular drug transporters (208). This transport defect is unrelated to hepatocellular injury or cholestasis and resembles reactions to cyclosporine.

Interstitial nephritis, described with intermittent use of rifampin, can accompany liver injury. Conversely, in one series of 60 patients with rifampin nephrotoxicity, concurrent hepatotoxicity was present in 25% (209).

Increased liver enzymes were reported in 12% of recipients treated with high-dose *rifabutin*, a rifamycin antibiotic. Serious liver injury has not been reported (185).

Pyrazinamide

Pyrazinamide causes dose-dependent liver injury. High-dose pyrazinamide (40 to 50 mg/kg) was associated with a greater frequency of hepatotoxicity than the doses used in current regimes (25 to 35 mg/kg). The concurrent administration of pyrazinamide with INH–rifampin increases the risk of hepatotoxicity compared to dual therapy (210). A recently approved 2-month protocol of rifampin and pyrazinamide for treating latent tuberculosis infection (LTBI) was associated with significant liver injury. Twenty-three cases of acute hepatitis were reported (211), all developing in the second month of treatment. There were seven deaths and the case-fatality rate was estimated to be 0.9 per 1,000 treatment initiations. In some cases, patients continued taking their medications after the onset of symptoms. On the other hand, one patient developed symptoms 6 weeks

after the drugs were stopped and eventually needed a liver transplantation 1 month later. Many restrictions have been placed in using this drug combination for LTBI; frequent liver test monitoring, absence of alcohol abuse or history of isoniazid toxicity, and need for monitoring by experienced physicians have been recommended (211). An alternative combination of pyrazinamide with levofloxacin for treating LTBI has also fallen out of favor because of the increased frequency of side effects, including hepatocellular injury (212).

Perceptions of the excellent safety of antitubercular drugs in children have been challenged by a Japanese report of hepatotoxicity in children younger than 5 years (213). Ohkawa et al. studied 117 pediatric recipients of antituberculosis treatment. Of these, 8% developed hepatotoxicity, all eight being younger than 5 years; four were receiving pyrazinamide. The probability of developing liver injury with a pyrazinamide-based regime was estimated as 0.95, 0.72, and 0.16 at 1, 5, and 10 years of age, respectively. On the basis of this observation, the authors have suggested careful monitoring of children younger than 5 years receiving pyrazinamide-based antituberculosis drug treatment (213).

Antifungal Drugs

Liver injury with the oral "azole" antifungal agents is best documented for *ketoconazole* (Table 33.20), but *itraconazole* and *fluconazole* have been implicated in a few reports (214,215,216,217,218,219,220,221). *Itraconazole* is largely perceived as being nonhepatotoxic. However, among more than 69,000 patients who received an oral antifungal agent, ketoconazole and itraconazole were most often associated with liver injury; the relative risks were 228 and 17.7 respectively, compared to nonusers (222). Further, a U.S. Food and Drug Administration (FDA) public health advisory has been issued after receiving 40 reports of acute liver failure in association with itraconazole; there were 22 deaths and two patients needed liver transplantation. In view of the serious nature of possible (although rare) liver injury, a diagnosis of onychomycosis should be clearly established before starting antifungal treatment.

Ketoconazole

Between 5% and 17% of recipients of ketoconazole develop abnormal liver test results. Previous estimates of symptomatic hepatitis have varied between 0.7 and 5 per 10,000 persons exposed (214,223). More recent studies cite figures of almost 20 per 10,000 persons exposed (222,224). Biochemical tests most often reflect a hepatocellular pattern, but cholestatic or mixed hepatocellular–cholestatic reactions are also common.

Women (female-to-male ratio 2:1) and persons older than 40 years are particularly susceptible (214,224) to ketoconazole-induced liver injury.

Table 33.20. Liver Injury Associated with Antifungal Drugs

Drug	Pattern of liver injury	Comments
Amphotericin	Hepatocellular	Rare
Griseofulvin	Cholestasis	Mallory bodies in mice; liver toxicity in humans is rare
Ketoconazole	Hepatitis, cholestasis, fulminant liver failure, cirrhosis (single case)	Frequency of liver injury is approximately 20 per 10,000 persons exposed
Itraconazole	Acute hepatitis, prolonged cholestasis (two cases) with biliary ductopenia (vanishing bile duct syndrome); acute liver failure	Pulse therapy appears safer than continuous dose regimes
Fluconazole	Raised alanine aminotransferase level in 25% of recipients, rarely hepatitis, fatal hepatic necrosis	Patients with human immunodeficiency virus/acquired immunodeficiency syndrome may be particularly susceptible
Terbinafine	Cholestasis, vanishing bile duct syndrome, acute liver failure	Frequency of liver injury approximately 1 per million persons exposed
Flucytosine	Transient rise in aminotransferase levels (10%); hepatic necrosis	Dose-dependent liver injury

Symptoms develop within 6 weeks in 60% of patients but can also be delayed (up to 6 months) (214,225). Jaundice occurs in 50% of those developing acute hepatitis, but up to one third may present with nonspecific symptoms, such as nausea, anorexia, and vomiting. Fever, rash, eosinophilia, and other immunoallergic characteristics are uncommon; liver injury is therefore hypothesized as a sequel of metabolic idiosyncrasy (226). Unintentional positive rechallenge has been described 30 months after the first reaction (227). Fulminant hepatic failure requiring liver transplantation has been reported (228). Fatal liver failure also follows the use of ketoconazole to reduce hypercortisolism in Cushing's syndrome (229).

The common histologic change is diffuse hepatocellular necrosis, particularly evident in zone 3, but cholestasis can be prominent (214). Recovery is usual after discontinuation of the drug, but progressive liver disease can occur if the drug is continued after the onset of symptoms. Several instances of protracted jaundice have been described. In one case ascites, pedal edema, and progressive hypoalbuminemia were observed after an initial episode of acute hepatitis; liver histology obtained after 6 months showed cirrhosis (230).

Terbinafine

Terbinafine is an allylamine derivative used to treat superficial onychomycosis. Elevation of AT level (>2 × ULN) is observed in 3.3% of those treated, but the frequency of symptomatic hepatitis is substantially lower (approximately 2 per 10,000 persons exposed) (231).

Hepatocellular mixed or bland cholestatic reactions have been reported. Most patients were older than 50. No clear gender differences were observed (232). Onset is within 3 to 8 weeks of beginning treatment. Symptoms may appear as long as 2 months after the drug has been discontinued; liver biopsies show hepatocyte degeneration and canalicular cholestasis with variable portal tract inflammation (82). Prolonged cholestasis with progressive biliary ductopenia and portal fibrosis (233,234) can occur. Terbinafine hepatotoxicity mimicking acute rejection has been reported in a liver transplant recipient (235). Although a rare association, the FDA has received at least 16 reports of fulminant liver failure possibly linked to terbinafine (236). Such an outcome has been estimated at 1 per million persons exposed (231). Recovery is usual with drug discontinuation. There have been anecdotal reports of ursodeoxycholic acid hastening recovery in a few protracted cases (237). Terbinafine-associated liver injury is probably idiosyncratic because hypersensitivity features are unusual. The recent identification of a hepatotoxic metabolite of terbinafine is consistent with this hypothesis (238). Liver injury associated with other antifungal drugs is summarized in Table 33.20.

Agents Used to Treat Parasitic Infestations

Pyrimethamine–sulfadoxine (Fansidar) has been linked with several instances of severe hepatocellular injury, including fatalities from hepatic necrosis. The frequency of liver toxicity is approximately 1 per 100,000 prescriptions (239). Because liver injury has been attributed to pyrimethamine and sulfonamides (see preceding text), it is not clear which component is responsible or whether interaction between the two components is important (240). The hepatotoxicity of other antimalarial and antiparasitic agents is summarized in Table 33.21.

Table 33.21. Liver Injury Associated with Antiparasitic Agents

Drug	Pattern of liver injury	References
Albendazole	Raised aminotransferase levels (20%), jaundice (5%)	(241)
Amodiaquine	Severe hepatitis (1 in 15,000 exposed); dose dependent	(239)
Antimonials	Steatosis	(2)
Artemisinin	Raised aminotransferase levels (0.9%)	(242)
Atovaquone-proguanil	Transient alterations in aminotransferase levels	(243)
Chlorguanide	Acute hepatitis (single case)	(244)
Mebendazole	Acute hepatitis; granulomatous hepatitis	(245)
Mefloquine	Transient rise in aminotransferase levels, fatty liver (one case)	(246,247)
Metronidazole	Hepatocellular carcinoma; cholestasis (few cases)	(248,249)

Ornidazole	Acute hepatitis, acute cholestatic hepatitis, chronic hepatitis	(250)
Thiabendazole	Cholestasis, vanishing bile duct syndrome; cirrhosis, occasionally associated with sicca syndrome	(251,252)
Quinine	Granulomatous hepatitis (usual presentation); mixed hepatocellular–cholestatic reactions	(253,254)
Quinacrine	Acute hepatitis, cholangitis	(255)

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Antiretroviral Therapy (Highly Active Antiretroviral Treatment)

The frequency of hepatic injury with HAART (which often includes three to four agents) is at least 10%. The agents used can be broadly categorized as nucleoside (or nucleotide) reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors, and protease inhibitors.

Nucleoside (or nucleotide) reverse transcriptase inhibitors

Mitochondrial toxicity is a group-specific feature of the NRTIs used to treat HIV (256,257) and HBV infections. The first-generation NRTIs, zidovudine, didanosine, and stavudine, have most often been implicated in causing liver injury (258,259,260,261,262,263,264,265). These agents inhibit mitochondrial deoxyribonucleic acid (DNA) polymerase-γ, a key enzyme involved in mitochondrial DNA replication. Additional mechanism of hepatotoxicity may involve oxidative stress, the consequences of impaired oxidative phosphorylation and fatty acyl β-oxidation, and insulin resistance. Reported risk factors for mitochondrial drug toxicity among people with HIV infection include obesity, female gender, and absence of AIDS-defining illness (256); one study was unable to confirm the gender predisposition or the influence of HIV status (256). Mitochondrial toxicity is unusual with second-generation NRTIs such as lamivudine, tenofovir, and abacavir.

The hallmarks of mitochondrial hepatotoxicity are extensive microvesicular and/or macrovesicular steatosis, lactic acidosis (sometimes with a shock-like state), and liver test abnormalities, progressing to acute liver failure. Asymptomatic hyperlactatemia is common (especially with stavudine) among those treated with HAART (266), but life-threatening lactic acidosis/hepatic steatosis is rare; the estimated risk is 1 to 15 per 1,000 person-years of antiretroviral use (259,267). Onset is usually within 6 months (range, 3 to 17 months) of starting treatment. The presenting symptoms are nonspecific: Nausea, vomiting, diarrhea, dyspnea, lethargy, and abdominal pain. In many cases there are extrahepatic manifestations, such as myopathy or peripheral neuropathy, and in severe cases pancreatitis and renal failure may follow the onset of the lactic acidosis and liver injury. Discontinuation of drugs is mandatory, but despite this deaths still occur (259).

Non-nucleoside reverse transcriptase inhibitors

Persons receiving nevirapine or *abacavir* (an NRTI) can develop an early (within 6 weeks) severe hypersensitivity reaction manifested by fever, rash, and eosinophilia. In such individuals, further use of these agents is contraindicated (Table 33.5). *Nevirapine* has also been implicated in causing severe hepatotoxicity (268,269). Examples include health care workers developing liver injury after receiving nevirapine as part of postexposure prophylaxis (270). The FDA received 12 reports of such hepatotoxic reactions during 1997 through 2000; seven recipients developed acute hepatitis, four had asymptomatic increases in serum AT levels, and one person developed acute liver failure. It is noteworthy that in some cases there was no adherence to the recommended 2-week dose escalation regime (271).

Nevirapine is no longer recommended for postexposure prophylaxis and should be used with caution in individuals with liver disease (272). Sequential toxicity with nevirapine followed by *efavirenz* has been reported in an HIV–HCV coinfected person (273). Although one study reported a lower frequency of liver toxicity with efavirenz (8%) as compared to nevirapine (15%), there are other reports that did not

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show significant differences in hepatotoxicity (267). A combination of genetic and immune factors has been identified that could predict some nevirapine reactions. In one predominantly white cohort, HIV-positive individuals with a CD4 percentage over 25% who also carried the HLA-DRB1*0101 haplotype were at risk of developing liver toxicity with rash and/or fever. The authors calculated that screening for this HLA haplotype could prevent 1 in 14 potentially fatal reactions (274) among whites with a CD4 percentage over 25%. Pregnant women should also use nevirapine with great caution. Likewise, the same group also found that the presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 had a positive predictive value of 100% in predicting abacavir hypersensitivity (275).

Protease inhibitors

Elevated liver enzyme levels are common with this class of drugs, but clinical hepatitis is infrequent (276).

The agents most often implicated in liver injury are *ritonavir* and *indinavir*. *Indinavir* and *atazanavir* may also be associated with unconjugated hyperbilirubinemia in 6% to 40% of treated persons, resulting from inhibition of uridine diphosphate–glucuronosyl transferase, a finding that is of no clinical consequence (277). Severe acute hepatitis can rarely occur with indinavir and atazanavir (278,279). The association with peripheral and/or tissue eosinophilia in some biopsied cases involving indinavir suggests an immunoallergic liver injury (279).

Acute hepatitis has also been reported in 3% to 30% of those prescribed ritonavir (280). The course of the illness is generally mild, and the liver injury responds favorably to drug withdrawal. Rarely, acute liver failure may develop; in these cases, liver histology shows severe microvesicular steatosis, cholestasis, and extensive fibrosis (280). By contrast, the combination of low-dose ritonavir (200 mg/day) with other protease inhibitors is not associated with an increased frequency of severe liver injury when compared with protease inhibitors such as nelfinavir or efavirenz (281).

Two studies have addressed the potential impact of underlying chronic viral hepatitis on the toxicity of protease inhibitors. Although hepatotoxicity was more frequent, the liver injury was rapidly reversible in most cases; this suggests that there is no overall detrimental effect of protease inhibitors when used in HIV/HCV or HIV/HBV coinfected persons (282). Further, the immune reconstitution that can follow successful HAART may cause a flare-up of previously quiescent chronic hepatitis B (283).

Monitoring for hepatotoxicity

The concept of monitoring for hepatotoxicity is beyond the scope of this chapter (see also HIV and the liver) but a few general rules have emerged. Baseline and serial monitoring of liver tests should be routine in HAART recipients. Grade 4 hepatotoxicity (AT × 10 × ULN or bilirubin × 5 × ULN) warrants immediate discontinuation and use of an alternative drug combination. For lesser changes in liver test results, frequent monitoring (1 to 2 weeks) is suggested, with treatment being continued only if there are no signs and symptoms of hyperlactatemia, hepatitis, or hypersensitivity (267).

Cardiovascular Drugs

Angiotensin-converting enzyme inhibitors

Drug-induced liver disease is a rare but important complication of this widely prescribed class of drugs. Onset of liver injury has varied from 2 weeks to 4 years after starting treatment (284). Cholestatic liver toxicity predominates. Recovery is usual but may be protracted (up to 6 months). Fatalities or advanced liver injury (e.g., ductopenia, cirrhosis) are infrequent (285).

Captopril is the oldest and possibly most hepatotoxic representative. Reactions to it and to *enalapril* usually manifest as cholestatic hepatitis, but hepatocellular or mixed hepatocellular reactions can occur both in adults and rarely in children (284). Enalapril-related cholestatic hepatitis can culminate in biliary ductopenia. However, the short-term outcome seems favorable after drug withdrawal (286). Hypersensitivity features such as fever, skin rashes, and eosinophilia can accompany captopril hepatotoxicity (287). Fulminant hepatic failure has been attributed to *lisinopril* (288), while *fosinopril* has been associated with bland cholestasis (289). Three instances of cholestatic liver injury have been recorded with *ramipril*; liver histology showed cholestasis, bile duct necrosis, bile extravasation, and ductular proliferation. Prolonged cholestasis was observed in one case (14 months); liver biopsy showed biliary cirrhosis (285).

Methyldopa

Methyldopa is much less prescribed now, but it retains a place in the management of obstetric hypertension. The history of methyldopa-induced liver injury remains relevant because of the range of hepatic disorders it can produce. These encompass a spectrum of toxicity including asymptomatic elevation of AT levels, acute hepatitis, chronic hepatitis, cirrhosis, bland cholestasis, cholestatic hepatitis, steatosis, and hepatic granulomas (14,290,291). Between 10% and 30% of recipients have abnormal liver test results without overt liver disease, and these changes often resolve despite continuation of the drug (2). Acute hepatitis occurs in less than 0.1% of recipients, usually within 3 months of starting methyldopa treatment; up to 50% present within 2 to 4 weeks.

The clinical presentation resembles that of acute viral hepatitis, with anorexia, nausea, and vomiting,

followed by jaundice. Serum AT levels are elevated (5 to 30 × ULN), with moderately raised SAP level. Liver biopsy specimens show focal or confluent hepatocellular necrosis, acidophilic bodies, and mixed portal/periportal inflammatory infiltrates. Bridging hepatic necrosis and massive hepatic necrosis can occur. Tissue or peripheral eosinophilia is rare. Most patients recover after withdrawal of the drug. However, mortality among patients presenting with jaundice has been reported to be 10% or higher (290).

Chronic hepatitis or cirrhosis has been reported as the first clinical manifestation of liver injury (290). In such cases, liver histology shows confluent areas of lobular collapse, periportal necrosis, and inflammation; the latter includes plasma cells and occasional eosinophils (290). Fibrous septa and established cirrhosis may also be present. Older reports of middle-aged women with steatosis and cirrhosis attributed to long-term methyldopa therapy may have confused drug reactions with NASH (108).

The pathogenesis of methyldopa-induced liver injury has been attributed to either immunologic or metabolic

idiosyncrasy; this dramatically illustrates the uncertainty of the indirect criteria used to assign etiopathogenic mechanisms of drug-induced liver disease. Female preponderance, positive rechallenge, presence of autoantibodies (ANAs, less commonly SMAs), hypergammaglobulinemia, and a positive Coombs' test have been cited in favor of an immune basis, but many of these features occur in those taking methyldopa without developing liver injury. Conversely, there is some evidence that methyldopa can be metabolized to a reactive intermediate (semiquinone or quinone) (2), and this could cause membrane injury or generate a neoantigen target for an immune-mediated hepatodestructive response.

β-Blockers

The hepatotoxic potential of β-blockers is very low, but reactions to *acebutolol*, *propranolol* (2), *metoprolol* (292) (both hepatocellular), *atenolol* (cholestasis), *carvedilol* (mixed hepatocellular–cholestatic) (293,294,295), and *labetalol* (296) have been described. *Labetalol* has been implicated in over 11 reports of acute hepatitis, including three fatal cases (296); histology showed massive, submassive hepatic necrosis or chronic hepatitis.

Calcium channel blockers

Liver injury associated with the calcium channel antagonists (e.g., *verapamil*, *nifedipine*, *diltiazem*) is infrequent. Most reported cases were hepatocellular in nature (297), but other presentations include cholestasis, granulomatous hepatitis (e.g., diltiazem) (298), and steatohepatitis (e.g., nifedipine and diltiazem) (299). As discussed earlier, the association of calcium channel blockers with steatohepatitis may be fortuitous because hypertension is a component of the metabolic syndrome, which predisposes to NASH.

Diuretics

Of the currently used thiazide derivatives, *chlorothiazide* (14), *chlorthalidone*, and *hydrochlorothiazide* (2) have been linked with rare instances of cholestasis. *Ticrynafen* (tienilic acid), a uricosuric diuretic, was associated with acute and chronic hepatocellular injury. The case-fatality rate was 10% (300), as a result of which this agent was withdrawn. Experimental studies of tienilic acid toxicity have been of interest because they provide an illustration of drug-induced AIH. Circulating antibodies directed against hepatic CYP 2C9 (which catalyzes the oxidation of tienilic acid) were often present in the sera of affected patients (301). There are a few anecdotal reports of acute hepatitis with *spironolactone* (14,302).

Hydralazine

Acute hepatitis with bridging necrosis is an occasional complication of hydralazine treatment. Granulomas (303) and cholestasis have also been described (304).

Other antihypertensive agents

Angiotensin II receptor antagonists

Although their overall hepatotoxic potential appears low, cases of liver injury have accrued with all drugs within this group. Most cases have occurred within 5 months of commencement. *Tasosartan* was withdrawn at the preregistration stage because of hepatotoxicity (305). *Losartan*, *valsartan*, and *candesartan* have been implicated in causing acute hepatitis or cholestatic hepatitis (306,307). Early resolution has been the rule after stopping these agents. Exceptions include two reports of prolonged cholestasis (1 year) after *irbesartan*-associated cholestatic hepatitis and biliary ductopenia accompanied by portal–portal fibrosis with candesartan (306). Irbesartan has also been implicated in triggering acute hepatitis with histologic features of AIH. Although autoantibodies were absent, the subsequent course, including steroid responsiveness and relapse with steroid withdrawal, was indistinguishable from AIH (308).

Antiarrhythmic Drugs

Amiodarone

Amiodarone is widely used in treating refractory ventricular and supraventricular tachyarrhythmias. Raised AT levels (one- to fivefold) are observed in up to 25%, but clinically significant liver disease occurs in 0.6%

of amiodarone recipients (309). The typical histologic lesion is a form of steatohepatitis that is indistinguishable from alcoholic liver disease (309,310), with microvesicular and macrovesicular steatosis, hydropic change, Mallory bodies, neutrophilic infiltrates, and pericellular and perivenular fibrosis. Micronodular cirrhosis is present in 15% to 50% (311) of cases. Other manifestations include granulomas, cholangitis (312), and acute liver failure; the latter may result from severe acute hepatitis or a Reye's syndrome–like illness (313), which has been reported within a few days of starting treatment with amiodarone. Amiodarone can also cause acute hepatic injury (314) within hours to days of an intravenous loading dose. Rapid resolution follows drug withdrawal, although a single loading dose can be fatal. In a few cases, positive rechallenge has been observed. The vehicle, polysorbate 80, rather than amiodarone itself may be responsible for the hepatotoxicity associated with parenteral preparations of the drug; the successful reintroduction of oral amiodarone in such cases supports this hypothesis (315,316).

The onset of amiodarone-induced chronic liver disease is delayed and insidious; most patients have received

treatment for at least a year (median, 21 months) (14). Symptoms include fatigue, weight loss, nausea, vomiting, and abdominal swelling due to ascites. Hepatomegaly, jaundice, and other features of chronic liver disease are evident. Liver test abnormalities are often seemingly minor, even in cases with hepatic decompensation. AT values are increased at least fivefold above normal, with the AST:ALT ratio close to unity; this differs from those with alcoholic hepatitis. There is a minor increase in SAP level. In more severe cases there may be jaundice, hypoalbuminemia, and prolongation of prothrombin time. Chronic liver injury from amiodarone is associated with prolonged storage of the drug in the liver, which therefore appears radio-opaque on computed tomographic (CT) examination because of the iodine content of this iodinated benzofuran derivative (317). Electron microscopy shows lysosomal inclusions (representing phospholipidosis) in hepatocytes and other liver cells (318). The presence of hepatocytes with the granular cytoplasm has been suggested as an early marker of amiodarone toxicity (319).

In less severe cases, biochemical resolution occurs between 2 weeks and 4 months after stopping amiodarone. However, in advanced cases the mortality is high, with a progressive decline in liver function despite discontinuing treatment. A likely explanation is that tissue levels of the drug remain high for some time because of the prolonged retention of amiodarone and its principal metabolite *N*-desethylamiodarone in hepatic lysosomes.

Prevention of amiodarone toxicity by baseline and serial monitoring of liver tests has been suggested (320). In many cases, this is impractical because of the high frequency of abnormal liver test results in patients treated with amiodarone because of factors such as cardiac failure, other drugs, or NASH. Furthermore, the development of abnormal liver test results during amiodarone therapy represents a conundrum because in one uncontrolled study the cardiac mortality after stopping amiodarone exceeded the total mortality among those who continued the drug despite suspected adverse reactions (311). Another recommendation is the measurement of serum amiodarone levels in long-term amiodarone recipients; ALT level elevations were minimal in those with serum amiodarone concentration of less than 1.5 mg/L (320).

The pathogenesis of phospholipidosis from amiodarone is unclear but may be related to inhibition of the lysosomal phospholipase, A_1 and A (321). Phospholipidosis is a constant association with steatohepatitis but appeared not to be related to it pathogenetically in animal studies. A series of elegant studies from Hospital Beaujon in Paris has shown that amiodarone, an amphiphilic cationic compound, accumulates in the anionic milieu of the mitochondrial matrix, and this leads to the disruption of mitochondrial electron transport and impairment of β-oxidation of fatty acids, resulting in microvesicular steatosis, generation of oxidative stress, and lipid peroxidation (322,323,324).

Other antiarrhythmic drugs

Quinidine is an infrequent cause of hepatocellular injury. Fever and elevated AT levels occur 1 to 24 weeks after commencing therapy (325). Liver histology shows hepatic necrosis and granulomas (326). Recovery usually occurs after stopping the drug. *Procainamide* has been associated with hepatic granulomas, hepatocellular injury, and intrahepatic cholestasis (14,327). *Propafenone* has been implicated in at least nine cases of predominantly cholestatic jaundice (328). Most reported cases occurred within 2 to 6 weeks (range, 2 to 28 weeks). Liver biopsy specimens showed portal tract inflammation with prominent cholangiolitis. No fatalities were recorded. Resolution occurred several months after drug cessation (328).

Anticoagulants

Unfractionated heparin and *low-molecular-weight heparins* can both cause elevation of serum AT levels (329). At least four cases of cholestasis have accrued with unfractionated heparin (329). Of the currently used oral anticoagulants, the *coumarin* derivatives (e.g., warfarin, phenprocoumon, dicoumarol, acenocoumarol) have been associated with transient increases in liver enzyme levels, which usually settle without changing therapy. Occasionally, they may cause hepatocellular or mixed hepatocellular–cholestatic injury and bland cholestasis (329). Several instances of acute

hepatitis including subacute liver failure requiring liver transplantation have been reported from Germany, where *phenprocoumon* is used extensively (330,331). An alternative coumarin or reintroduction of phenprocoumon can be considered in mild cases. However, reintroduction may be unsuccessful and further cross-reactivity with other coumarins can also occur. In such cases, long-term anticoagulation is achieved with low-molecular-weight heparin. *Phenindione* has now been withdrawn; it was associated with severe hypersensitivity reactions and liver injury in up to 10% of recipients (14).

Lipid-Lowering Drugs

Hydroxymethylglutaryl-coenzyme A reductase inhibitors (the "statins")

Overall, the statins are not strongly associated with significant hepatic injury, although there appears to be discordance between literature reports and data contributed to drug safety surveillance authorities. The frequency of hepatotoxicity is estimated at approximately 1 per 100,000 patient-years of exposure; comparative figures for NSAID liver injury are between 2.2 and 50 per 100,000 patient-years of exposure (332). Between 1% and 3% of people who receive statins develop a dose-related increase in ATs (332). These abnormalities settle rapidly with discontinuation of treatment and often also if therapy is not interrupted (332). Further, a recent study did not find significant differences in the frequency of severe hepatoxicity (serum bilirubin >3 mg/dL or AT × 10 times ULN or baseline values) between a cohort with and without

baseline elevation of liver test results (0.6%, 0.2%, respectively) (333).

Lovastatin (334), *pravastatin* (335), *atorvastatin* (336), and *simvastatin* (337) have been implicated in a few cases of cholestatic hepatitis. Acute liver failure has also been attributed to lovastatin. This is an extremely rare event (<1 per million patient-treatment years) and its frequency is similar to the background rates for idiosyncratic acute liver failure in the community. AIH has been associated with atorvastatin; the drug appears to have unmasked preexisting AIH rather than induce immune liver injury by itself (338).

Although monitoring liver enzyme levels is often recommended, this is neither likely to predict toxicity (331,339) nor likely to be cost-effective. In the Air Force/Texas Coronary Atherosclerosis Prevention (AFCAPS/TEXCAPS) study, only 18 of 100,000 AT estimations performed were raised more than three times the ULN; in none of these instances did the affected person develop hepatitis (339). Like amiodarone, the benefits of continued treatment may outweigh the potential (but low) risks of liver injury.

Other lipid-lowering drugs

Rare cases of acute hepatitis and cholestatic hepatitis have been attributed to the *fibrates*. A few cases of chronic hepatitis with portal or bridging fibrosis have also been described in persons receiving statins (340). Serologic and histologic features resembling AIH were observed but these resolved after the drugs were discontinued. Other than one report of hepatocellular injury with *colestipol*, bile acid sequestrants have not been implicated in causing liver injury (341).

Nicotinic acid (niacin)

The hepatotoxic potential of nicotinic acid has been recognized for more than 60 years; it is a dose-dependent hepatotoxin. Liver injury usually occurs at dosages that exceed 2 g/day, but rare examples of low-dosage (500 mg/day) sustained release (SR) niacin have been implicated in fulminant hepatic failure (342). Patients taking sulfonylurea drugs and those with preexisting liver disease, particularly alcoholic hepatitis, are at increased risk of nicotinamide hepatotoxicity (343,344).

Both the crystalline unmodified immediate-release (IR) and SR preparations have been associated with liver injury (345,346,347). SR preparations have improved bioavailability and are therefore more potent (about twice) than equivalent IR preparations; a dose reduction of 50% to 70% has been recommended (347). Hepatotoxicity has often occurred when the formulation of nicotinamide was changed without appropriate dose modification.

The symptoms of nicotinamide hepatotoxicity begin as early as 1 week to as long as 4 years after initiating therapy. Fatigue, malaise, anorexia, and jaundice are typical features, usually resolving completely when the drug is stopped. Liver biopsy specimens show hepatic necrosis and centrilobular cholestasis. Well-documented cases of fulminant hepatitis have also been attributed to nicotinamide, some necessitating liver transplantation (346).

Niaspan is an extended-release preparation of nicotinamide with IR characteristics. Its hepatotoxic potential appears to be minimal. Less than 3% of recipients developed elevated AT levels greater than two times the ULN; most were also receiving statins. Liver test results normalized with dose reduction (348).

Drugs Used in the Treatment of Endocrine Disorders

Oral contraceptive and anabolic steroids

Both OCSs and the 17-alkylated anabolic steroids are associated with cholestasis, vascular lesions, and hepatic neoplasms. However, the strength of these associations with individual lesions varies. Benign hepatic neoplasms are clearly associated with OCS, whereas

their association with HCC is controversial. By contrast, HCC is well documented in users of anabolic steroids. Likewise, hepatic and portal vein thrombosis is an established adverse effect of OCS, but not with anabolic steroids. Other vascular lesions, such as peliosis hepatis, are more often observed with anabolic steroids than with OCS.

Oral contraceptive steroids

Cholestasis

The frequency of cholestasis with OCS is 2.5 per 10,000 women exposed. Genetic factors influence the frequency of this complication, with a particularly high incidence among women in Chile and Scandinavia. Individuals with a previous history of cholestasis of pregnancy are also at risk (50%). Mild prodromal symptoms such as anorexia and nausea, followed by pruritus, develop 2 to 3 months after starting OCS (rarely as late as 9 months). SAP level is moderately elevated, and this is accompanied by a transient increase in ATs, occasionally exceeding 10 × ULN; GGTP levels are often normal. Development of chronic cholestasis is an extremely rare occurrence (82) and, in general, recovery is invariable within days to weeks of drug cessation. Hormonal replacement therapy (HRT) is safe in patients with liver disease, but jaundiced patients may experience an increase in bilirubin levels and should be monitored with liver tests (82).

Benign neoplasms

OCSs can induce enlargement of preexisting *hemangiomas* through their trophic effects on the vascular endothelium (349). Recurrences of hemangiomas have also been described in patients with a history of previously resected lesions (350). A role for estrogens in the pathogenesis of FNH is plausible because these lesions occur principally in young women (up to 86%) (351). However, unlike liver adenomas, an increased incidence of FNH has not been observed commensurate with the increasing use of OCS. Further, FNH can occur in persons not using OCSs (352). A study of over 200 patients with FNH failed to show a relationship between OCS and the size or number of FNH lesions (352). Discontinuation of OCS did not generally influence the size of FNH, and estrogen-dependent tumors were rare. The authors suggested that hepatic adenomas misclassified as FNH, could account for the apparent growth of FNH reported previously with OCS.

The association between OCS and *hepatic adenomas* was described approximately 30 years ago. The increasing frequency of this neoplasm has paralleled the rising use of OCS. In the 1970s and 1980s, the annual risk was approximately 3 to 4 per 100,000 exposed persons (25,100), but this risk is now probably lower with the lower-dose preparations currently used. There is also a relationship with the duration of OCS use, so that the risk (compared to nonusers) for long-term OCS users (>10 years) is increased 100-fold. Patients with liver adenomas usually present with a painless or tender right upper quadrant mass or occasionally with hemoperitoneum secondary to hepatic rupture. Adenomas usually regress after OCSs are withdrawn (101), but surgery is required for larger lesions to avert possible rupture (353) or because of the small but definite risk of malignant transformation. To prevent hepatic adenomas, OCSs with lower estrogenic potency are preferred. Long uninterrupted periods of OCS use should be avoided.

Hepatocellular carcinoma

The relative risk for HCC is increased twofold among women who have ever taken OCS, and it is increased sevenfold in long-term users (>8 years) compared with age-matched controls (26,353,354). However, it must be emphasized that estrogen-related HCC is rare, accounting for less than 2% of primary liver cancer in western countries. In countries with a high prevalence of HCC, OCS is not an independent risk factor because of the far greater importance of chronic viral hepatitis and aflatoxin (26). Median age at presentation is 30 years, and HCC occurs at least 5 years after starting combination OCSs. The tumors are well differentiated and have a better short-term outcome than HCC because of other causes (14).

Anabolic steroids

Cholestasis

At high doses, anabolic steroids often produce reversible bland cholestasis, usually within 1 to 6 months of beginning treatment. Prolonged jaundice with ductopenia is a rare complication (355). Acute hepatitis is also an unusual sequel after self-ingestion of high-dose anabolic steroids (356).

Benign neoplasms

The association with hepatic adenomas is less robust. Many reports were from patients with Fanconi's anemia (357), a disorder caused by genomic instability and a resultant high background incidence of neoplasms. However, reports of adenomas among female body builders and in persons taking anabolic steroids for other indications (354,358) indicate a probable etiologic association. Oral and parenteral androgens are both implicated (354).

Hepatic adenomas were identified in 3 of 11 long-term users of *danazol* (359), all patients with hereditary angioneurotic edema. Danazol-associated hepatic adenomas have also been observed in other disorders such as SLE and endometriosis. For this reason, surveillance ultrasonography is suggested in long-term recipients of danazol (359) and also for former users of other anabolic steroids; liver tumors have been described up to 24 years after steroid discontinuation (354). These tumors can regress with androgen withdrawal (360), but this is not always the case and surgical resection may be required.

Hepatocellular carcinoma

Several cases of HCC have been documented (361,362), but confounding

factors such as viral hepatitis were not clearly excluded in earlier cases. Because some of these tumors metastasize late and sometimes regress (363), doubts have been expressed about the true nature of these tumors; it is sometimes difficult to distinguish well-differentiated HCC from adenomas (364). Furthermore, hepatic adenoma and HCC can coexist (365). Four reports of HCC have been attributed to long-term *danazol* use (366,367); the underlying diseases were hereditary angioneurotic edema, SLE, and chronic idiopathic thrombocytopenic purpura. These liver tumors were large, well-differentiated carcinomas that did not show regression with drug withdrawal. Serum α-fetoprotein levels were normal in three of the four cases; therefore, α-fetoprotein cannot be used as the sole tumor marker for diagnosis in these patients.

Estrogen-receptor antagonists

The range of *tamoxifen* hepatotoxicity includes hepatic steatosis, NASH, and rare instances of submassive hepatic necrosis and even cirrhosis. Of these, the association with hepatic steatosis and NASH is most striking. In one series of 66 women with breast cancer who had received tamoxifen for 3 to 5 years, 24 showed radiologic evidence of hepatic steatosis (368). In another study, liver biopsy specimens were obtained from 15

women with moderate-to-severe hepatic steatosis (as designated by liver/spleen CT scan ratio of <0.5); 14 showed NASH (369). The metabolic profile of these women resembled that of most patients with NASH; half were obese and hepatic steatosis correlated with increases in body mass index (370). Tamoxifen can also induce hypertriglyceridemia, a risk factor for NASH. It therefore seems possible that tamoxifen may play a synergistic role with other factors such as hyperlipidemia, obesity, and insulin resistance to cause NASH. A more direct role for tamoxifen has been postulated recently on the basis of results of a study showing inhibition of intrahepatic fatty acid synthesis, which contributes to steatosis (371).

Physicians should be aware of the high frequency of hepatic steatosis (approximately 30%), as determined by hepatic imaging, or NASH in women treated with tamoxifen. Monitoring patients for this adverse effect should include physical examination for hepatomegaly and liver tests. Some authors have also recommended that hepatic imaging (ultrasonography or CT) be performed annually (372). Liver biopsy is required to establish the severity of the disorder, particularly if liver test abnormalities fail to resolve after stopping tamoxifen, and also to exclude metastatic breast cancer in difficult cases. Many cases improve after stopping tamoxifen, but this is a valuable agent for the treatment of breast cancer, and it is not yet clear whether treatment should always be withdrawn permanently. Preliminary experience with bezafibrate appears promising; improvement in liver imaging characteristics and AT level was demonstrated in a small series (369). An alternate strategy is to attempt optimization of the metabolic milieu, especially in patients with preexisting hepatic steatosis; 43% of this group developed increases in AT levels with tamoxifen and were approximately three times more likely to have abnormal glucose tolerance as compared to those who did not (69% and 24%, respectively) (370). Similar conclusions were also reached in a recent Italian study, which found that obese and overweight women with features of the metabolic syndrome were at an increased risk of developing NASH with tamoxifen (373).

Toremifene, a tamoxifen analog, is also implicated in causing steatosis or steatohepatitis, but with a lower frequency (<10%) (372).

Antithyroid Drugs

The estimated frequency of hepatotoxicity from antithyroid drugs is less than 0.5% (374). *Methimazole* and *carbimazole* have rarely been incriminated in reports of cholestasis or cholestatic hepatitis (375,376). By contrast, *propylthiouracil* reactions usually result in acute hepatitis (374,377), which can be severe. Hypersensitivity features are frequently present. Histologic appearances range from portal inflammation and cholestasis in milder forms of hepatitis to submassive or massive hepatic necrosis in severe cases (378). Chronic hepatitis is a rare complication. A review of 36 published cases of liver toxicity associated with propylthiouracil identified seven deaths from acute liver failure (339,343). Three more fatal cases, including 1 in pregnancy, have been recently added to this list (379). Most patients recover, but continued ingestion of the drug after symptoms develop can lead to a poor outcome.

Anesthetic Agents

Halothane

In the first years after the introduction of halothane in the mid-1950s, most cases of postoperative massive liver necrosis were readily attributable to nonanesthetic causes, such as shock and hypoxia. However, cases of *unexplained massive hepatic necrosis after anesthesia* appeared to be more common after halothane than after the use of other agents (380). The clinical syndrome was characteristic, and the temporal relationships between exposure to halothane and the development of liver injury were convincing. This evidence, together with the observations of recurrence after deliberate or inadvertent rechallenge eventually led to the acceptance of *halothane hepatitis* as a real

entity. Rare cases of halothane-induced liver injury have also occurred after workplace exposure among anesthetists, surgeons, nurses, and laboratory staff, and after halothane sniffing for "recreational" use. Despite its rarity, the severity of cases of halothane hepatitis and the greater availability of equally acceptable agents have contributed to the virtual abandonment of halothane.

Two types of postoperative liver injury occur after the use of halothane. Minor elevations of the levels of ALT occur between the first and tenth postoperative day in 10% to 20% of patients, all of whom remain asymptomatic; the risk of hepatic enzyme abnormalities is higher after a second halothane anesthetic than for agents such as enflurane, isoflurane, and desflurane (14,381). This change in liver test results is rapidly reversible. Its relationship with the more severe lesions of halothane hepatitis is unclear; potentially the changes could be explained by minor nonspecific toxic injury or by ischemia-reperfusion consequent to the profound reduction of hepatic blood flow that can be ascribed to halothane anesthesia. Halothane hepatitis is a rare, dose-independent hepatic drug reaction. After one halothane anesthetic, the frequency of this reaction is very low (approximately 1 per 10,000 persons), but after two or more halothane exposures within a 28-day period, this increases to 15 per 10,000 exposed persons (14).

The reaction is unrelated to the type of surgery, duration of anesthesia, or to the presence of underlying liver disease. Halothane hepatitis is extremely rare in children, and more severe in those older than 40 years. Two thirds of cases are in women. A key feature is repeated exposure to halothane within a relatively short period; this is observed in 80% of cases. Further, many patients give a history of previous unexplained, delayed-onset fever, nausea, or jaundice in the postoperative period. Onset of liver injury is earlier after a repeat administration of halothane and typically increases in severity with each exposure. Obesity is another risk factor, possibly related to increased storage of halothane or induction of hepatic CYP 2E1, an enzyme involved

in halothane metabolism. The induction of CYP enzymes has been implicated experimentally in the pathogenesis of halothane-induced liver injury, and one study indicated that the coadministration of antiepileptic drugs predisposed patients to halothane hepatitis (382). A genetic predisposition to halothane-induced liver injury is evident in guinea pigs, and there are data in humans to invoke familial predisposition, such as instances of several closely related family members experiencing halothane hepatitis (10) Likewise, with the use of an in vitro test that detected injury to peripheral blood mononuclear cells after exposure to phenytoin epoxide, increased susceptibility was noted among the relatives of patients with halothane hepatitis, as well as in the patients themselves (383).

Fever is common in the first 48 hours after any major surgery, but fever associated with an adverse reaction to halothane is typically delayed until 3 to 14 days; occasionally, a rash is noted. Jaundice occurs within 21 days of halothane exposure; the median time to onset is 9 days after a single anesthetic and 5 days after multiple exposures. Jaundice is usually preceded or accompanied by symptoms of hepatitis. The liver may be swollen and tender, but in severe cases it decreases in size as a result of extensive hepatic necrosis. Liver failure ensues, with bruising, bleeding, clouding of consciousness and onset of hepatic coma, or hepatorenal failure. Renal failure may develop as part of the hepatorenal syndrome, but acute tubular necrosis could result from a nephrotoxic effect of halothane, as has been better documented for methoxyflurane. Liver histology may show zonal, bridging, or panlobular necrosis.

In milder cases, symptoms may not be attributed to a halothane reaction. It is therefore crucial to take a full history of earlier anesthetic exposures to prevent fatal cases of halothane-induced liver injury. In more severe cases, hepatic failure may follow a fulminant course. Apart from acetaminophen hepatotoxicity, halothane was the leading cause of drug-induced liver failure until the mid-1980s. The reported mortality is 10% to 80%, but the higher rates reflect referral to specialized centers (12,384,385,386). In most cases, however, symptoms resolve within 5 to 14 days and recovery is complete. Rare cases have been reported in which halothane was implicated as the cause of chronic liver disease (6,13,387).

Although an immunologic mechanism has been invoked as part of the causative mechanism (5,6), immunosuppressive agents do not alter the clinical outcome. Management centers on intensive medical support. Liver transplantation must be considered in cases with a poor prognosis, as presaged by previous episodes, early onset, serum bilirubin level of more than 200 µmol/L (10 mg/dL), and prolongation of prothrombin time. In adults, halothane-induced liver disease can be prevented in 90% of reported cases by paying attention to the previous history and adhering to safety guidelines. As many as two thirds of cases occur in individuals with a history of previous reactions to halothane, and most cases are associated with repeated use of halothane within 28 days, especially in obese middle-aged women.

Because halothane may leach out of the tubing of anesthetic devices, prevention of recurrence in sensitized patients requires that the equipment used for anesthesia should never have been exposed to halothane. Cross-sensitivity between halothane and other haloalkane anesthetics is best documented for

methoxyflurane, an agent that is no longer used because of nephrotoxicity. Cross-sensitivity is likely with enflurane and is possible for isoflurane, as described later, but it has not been reported for desflurane and sevoflurane.

There is no readily available diagnostic test for halothane hepatitis. Some interest has surrounded use of an antibody test claimed to be 80% sensitive (7), but independent validation is lacking and the test is not widely applicable. Diagnosis therefore requires careful consideration of the present and past relationships of liver injury to halothane exposure and rests also on the exclusion of other causes of liver disease in the postoperative period (see Chapter 25).

Other agents

General anesthetic agents in current use other than halothane have rarely been associated with postoperative liver failure and massive hepatic necrosis. However, the evidence that such agents cause idiosyncratic hepatic drug reactions is strong only for enflurane (14,388,389). The likelihood that individual haloalkane anesthetics can cause liver injury appears to be related to the extent to which they are metabolized by hepatic CYP enzymes, which is 20% for halothane, 2% for enflurane, 1% for sevoflurane, and 0.2% or less for isoflurane and desflurane. Accordingly, the estimated frequency of enflurane-related hepatitis is much less than that for halothane. The clinical syndrome is similar, with onset of fever within 3 days and jaundice in 3 to 19 days. At least one case was proved by positive rechallenge (388). Two thirds of patients had been previously exposed to either enflurane or halothane.

The possibility that *isoflurane* could be responsible for drug-induced liver injury is more contentious. More than 50 suspected cases were reported to the FDA by 1986, but in two thirds of these another potential cause of liver injury seemed more likely (389), and in the remainder isoflurane was only one of several possible factors that could cause hepatic damage. There have been a small number of more recent case reports in which isoflurane seemed to be the likely cause of fatal hepatotoxicity, either because of repeated exposure to isoflurane in the absence of other potential causes of liver injury (390,391,392) or because isoflurane had been administered after possible previous sensitization to halothane (393) or enflurane (394). Isoflurane should be regarded as a possible but very rare cause of hepatotoxicity; the pathogenic mechanism remains unclear.

The newer haloalkane anesthetics, *sevoflurane* and *desflurane*, appear essentially free from adverse hepatic events, although rare isolated reports have noted an association between liver injury and desflurane

anesthesia; none of these was proved by rechallenge (395).

Drugs Used in the Management of Diabetes Mellitus

Thiazolidinediones

Troglitazone

Troglitazone is a peroxisome proliferator activator receptor-γ agonist that was associated with elevated AT levels in 0.5% to 1.9% of recipients in a clinical trial (396). Reports of acute liver failure emerged early in the postmarketing phase (397,398,399). Troglitazone was implicated in over 75 instances of fatal hepatotoxicity or liver failure requiring hepatic transplantation (400) before it was withdrawn in 1999. The frequency of acute liver failure was estimated at 240 per million person-years of troglitazone exposure (3,400,401).

Troglitazone is metabolized by the CYPs 3A4 and 2C8. Unlike the newer thiazolidinediones, it has a unique α-tocopherol side chain that is metabolized to a quinone. This raises the possibility of quinone-related liver injury, similar to that of acetaminophen or methyldopa hepatotoxicity. However, the quinone metabolite is less toxic than the parent compound, and an alternative hypotheses implicating (402) mitochondrial injury leading to cell death (through necrosis or apoptosis) is currently favored (402,403). A potentially aggravating factor is bile salt–induced apoptosis resulting from inhibition of the bile salt export pump (Bsep) by troglitazone or by coadministration of cholestasis-inducing drugs such as glibenclamide (402,404). Abrogation of inherent cytoprotective mechanisms (e.g., reduction in heat shock protein 70 levels) may also contribute (405). Finally, features of drug allergy are unusual (396). Taken together, the existing data are consistent with an idiosyncratic metabolic form of liver injury. However, the evidence for any of these pathways is weak and/or indirect and speculative, as reviewed elsewhere (401,402).

Risk factors for troglitazone hepatotoxicity have not been clearly defined. The reported cases were generally in older women and obese persons, but this represents the common phenotype of those with type 2 diabetes; detailed epidemiologic studies have not been performed (377). In Japan, persons carrying mutations of CYP 2C19 were over-represented (50%) in a small series of eight patients with troglitazone-induced hepatocellular injury as compared to those without liver injury (13%) (406). Likewise, Japanese subjects carrying the combined glutathione-*S*-transferase GSTT1-GSTM1 null phenotype were three times more likely to develop liver injury. However, this phenotype was present in only approximately 21% to 59% of those developing liver toxicity, suggesting that other factors may be important (407). There is no evidence that preexisting liver disease or other drugs predispose to troglitazone hepatotoxicity, although a progressive

course reported in one patient was attributed to concurrent simvastatin and troglitazone (408). Interaction with NASH, which occurs in at least 20% of obese individuals with type 2 diabetes, has not been explored thoroughly (401). However, many patients affected by troglitazone-induced liver injury had normal liver test results before starting troglitazone treatment.

The onset of troglitazone hepatotoxicity was often as long as 9 months, and sometimes more than 12 months after starting treatment (409,410); rare cases have had a much earlier onset (3 days) (411). Patients presented with nausea, fatigue, jaundice, vomiting, and symptoms of liver failure. Progression from jaundice to encephalopathy, liver failure, or death was often rapid (average 24 days) (411), even after detection of biochemical abnormalities before the development of symptoms, and in some cases deterioration continued despite cessation of troglitazone intake (398). Only 13% of the patients survived without receiving a liver transplantation (411). Histology from biopsy specimens, explants, or autopsies showed submassive or massive hepatic necrosis, with postcollapse scarring, bile duct proliferation, and some eosinophils (412). Severe cholestasis has also been reported (413), but this is sometimes observed in other cases of fulminant hepatic failure (e.g., valproate) and does not necessarily imply any different pathogenic mechanism.

Rosiglitazone and pioglitazone

By comparison with troglitazone, serious liver injury is infrequent with these second-generation thiazolidinediones. In clinical trials, an elevation in the level of ALT (>3 × ULN) was reported in 0.25% and 0.26% of patients treated with rosiglitazone and pioglitazone, respectively (414). Rosiglitazone has been associated with six reports of hepatotoxicity (415,416,417). The onset was earlier (1 to 3 weeks) than that for most cases reported with troglitazone. Rarely, liver injury can occur beyond 1 year of use (418,419,420). Liver histology from two patients showed cholestatic hepatitis (420) or granulomatous hepatitis (418). Resolution occurred within 3 months in all but one fatal case. The validity of the diagnosis has been questioned in three of these cases (416,417,419). In one case, an alternative diagnosis of ischemic hepatitis was proposed because of the exceptionally high levels of ALT (11,000 U/L) and rapid resolution (within 9 days). In the other two reports, potential confounding factors were present such as alcohol ingestion or coprescription of known hepatotoxic agents such as acetaminophen, zafirlukast, and testosterone.

Pioglitazone has been implicated in five reports of acute hepatocellular injury (414,421). In most cases, liver injury occurred within 1 to 7 months of starting the drug and resolved within 3 months of discontinuing pioglitazone (414). Liver histology from two biopsied cases showed cholestatic hepatitis with bile duct injury (422). Other presentations of pioglitazone hepatotoxicity include one report of fulminant hepatic failure (421). Another instance of isolated, reversible increase in alkaline phosphatase likely represents adaptation (423).

The FDA recommends liver tests before beginning treatment with pioglitazone or rosiglitazone; pretreatment

ALT level should be less than 2.5 × ULN. Further evaluation every 2 months during the first year of therapy is recommended, and periodically thereafter. If ALT levels remain persistently elevated (>3 × ULN), the drug should be discontinued. As with INH, symptoms suggestive of hepatitis should be assessed immediately. Individuals who developed jaundice with troglitazone should not receive these thiazolidinediones, although cross-toxicity was not a problem in one reported case (420).

It must be noted that however intuitive such biochemical screening may seem, this protocol has not been validated. Even if adhered to strictly, it could not have prevented some cases of fatal hepatotoxicity in which transition from normal AT to liver failure occurred within 1 or 2 weeks (411). In this situation, the probability of detecting new cases by monthly monitoring is extremely low (estimated at 0.000065) (424). Further, the troglitazone episode has highlighted the low compliance with monitoring recommendations; less than 5% of patients taking troglitazone were monitored per protocol. Finally, the positive predictive value of abnormal liver tests is likely to be suboptimal in patients with type 2 diabetes (425) because of confounding factors such as nonalcoholic fatty liver disease (NAFLD)/NASH (see Chapter 39), chronic hepatitis C, or concomitant drug therapy.

Oral hypoglycemic drugs

Liver injury (typically hepatocellular) was common with older sulfonylureas, such as *carbutamide*, *metahexamide*, and *chlorpropamide* (2,14). Of the currently used agents, *tolbutamide*, *tolazamide*, *glibenclamide*, and *glimepiride* have rarely been associated with cholestasis or cholestatic hepatitis (2,426,427,428). Considering the structural relationship of sulfonylureas with sulfonamides, it is perhaps not surprising that hypersensitivity phenomena (fever, skin rash, eosinophilia) were present in some (but not all) cases (429). Most cases resolved after drug withdrawal, but chronic cholestasis progressing to VBDS has been described with tolbutamide and tolazamide (14). Death from liver failure has been reported in two patients, one of whom had cirrhosis (429). There are three reports of acute hepatitis induced by *gliclazide*; hypersensitivity features were present in one case (429a). *Glibenclamide* has also been

associated with hepatocellular liver injury and hepatic granulomas (426).

Table 33.22. Liver Injury Associated with Drugs Used in Diabetes Mellitus

Drug	Pattern of liver injury	Comments
Acarbose (glucosidase inhibitor)	Acute hepatitis; most reported cases from Spain (to date) Cholestasis with voglibose (another glucosidase inhibitor)	Positive rechallenge
Metformin (biguanide)	Cholestatic hepatitis; cholestasis; acute hepatitis	Very rare
Human insulin	Mixed liver injury; all reports from Japan; resolved when changed to porcine insulin	Positive rechallenge
Sulfonylureas	Acute hepatitis, cholestasis, cholestatic hepatitis, vanishing bile duct syndrome, granulomatous hepatitis	Usually reversible but fatalities reported
Repaglinide	Acute hepatocellular injury	Single report
Thiazolidinediones		
Troglitazone	Submassive or massive hepatic necrosis; cholestasis	Many fatal cases. Withdrawn from use
Rosiglitazone	Acute cholestatic hepatitis, granulomatous hepatitis	Few reports, fatalities rare
Pioglitazone	Acute hepatocellular injury; cholestatic hepatitis (one case), fulminant hepatic failure (one case)	Few reports, fatalities rare

Other antidiabetic agents that have been rarely associated with liver injury include *metformin*, *repaglinide*,

acarbose, and *human insulin* (430,431,432,433) (Table 33.22).

Analgesics and Drugs Used to Treat Rheumatologic Diseases

Acetaminophen

Acetaminophen (paracetamol) hepatotoxicity is an important cause of drug-induced liver injury in most countries (434,435); currently it accounts for approximately 50% of cases of acute liver failure in the United States (436). When used in recommended doses (1 to 4 g/day), acetaminophen is extremely safe (437), but single doses exceeding 15 to 25 g may cause severe liver injury that is fatal in up to a quarter of cases. Acetaminophen hepatotoxicity usually follows deliberate self-poisoning in an attempted suicidal or parasuicidal gesture. However, up to 30% of cases of acetaminophen hepatotoxicity admitted to hospital now result from "therapeutic misadventure," in which the daily dose has not greatly exceeded the recommended safe limits but in which specific risk factors were present (438,439) (see subsequent text); daily doses of 2 to 6 g have been associated with fatal hepatotoxicity. Cases associated with actual therapeutic doses are much rarer and may represent inadequate/unreliable disclosure and documentation.

Risk factors

Acetaminophen causes dose-dependent liver injury but individual susceptibility is also important. Therefore, death has occurred after ingestion of single doses of 7.5 g in adults or 150 mg/kg in children, whereas survival has been recorded with massive overdoses (50 g or more) (440). Age alters individual susceptibility; liver injury has been described even in neonates, but children are considered to be relatively resistant to acetaminophen poisoning (441). This has been attributed to differences in disposition and metabolism of the drug, but relatively larger liver and kidney sizes (as a proportion of total body weight) is an alternative explanation (442). However, there is increasing recognition that both intentional and unintentional acetaminophen toxicity can occur in children. Common prescribing errors involved in these cases include use of adult doses, wrong dosing intervals, concomitant use of other acetaminophen-containing or hepatotoxic products, and host factors—particularly undernutrition, fasting, and drug–drug interactions. Rectal preparations of acetaminophen were implicated in a few cases. The bioavailability of acetaminophen in this formulation can vary (up to ninefold). Furthermore, the slower onset of action encourages repetitive use and consequent cumulative toxicity (443). Although acetaminophen self-poisoning is more common in women, fatal acetaminophen hepatotoxicity occurs more frequently in men; this is largely due to alcoholism and late presentation (20).

Clinical features

The clinical evolution of liver injury follows three phases. In the first, anorexia, nausea, and vomiting are prominent, and this may last from 12 to 24 hours. These symptoms often subside so that the person often feels well during the second phase, which lasts another 24 hours. Signs of hepatic failure, often with renal insufficiency, appear 48 to 72 hours after ingestion of acetaminophen (phase 3). Pain over the liver may be pronounced. It is accompanied by jaundice, hypoglycemia, coagulopathy, renal failure, acidosis, and encephalopathy. Myocardial injury has also been described (440). Renal failure can occur with or without significant liver injury. In untreated subjects, death

occurs between 4 and 18 days after drug ingestion, usually from cerebral edema, and/or sepsis from multiorgan failure.

ALT levels are markedly elevated (2,000 to 10,000 IU/L); indeed, when the cause of acute liver injury is unclear (e.g., in an unconscious individual or someone known to have chronic hepatitis C), such high AT levels should arouse suspicion of acetaminophen toxicity. These high ALT values are unusual in viral hepatitis but may occur with ischemic hepatitis and with other forms of drug-induced liver injury, including herbal toxicity (see later section). The following clinical and laboratory indices predicate a poor outcome of acetaminophen hepatotoxicity: Prothrombin time greater than 100 seconds, serum creatinine greater than 300 μmol/L, the single finding of a pH of less than 7.3 after adequate fluid replacement, or grade 3 or 4 encephalopathy in patients with a normal pH (434,444). Other prognostic indices proposed include the Acute Physiology and Chronic Health Evaluation (APACHE) II score, and blood lactate, phosphate, and α-fetoprotein levels (445). None of these indices has been widely adopted, and they await independent validation.

Histology

The histologic features are zone 3 necrosis, with submassive (bridging) or panacinar (massive) necrosis in severe cases (14). Inflammatory activity is inconspicuous, and resolution occurs without fibrosis. Chronic liver injury has been described in patients consuming moderate doses of acetaminophen for many months (2 to 6 g each day). Preexisting liver disease or concurrent alcohol intake were not always excluded in such individuals (14), and the rapid resolution after discontinuing acetaminophen abuse implies a form of chronic hepatotoxicity rather than a syndrome of drug-induced chronic hepatitis.

Acetaminophen toxicity with therapeutic doses (therapeutic misadventure)

Enhanced susceptibility to acetaminophen toxicity is now well recognized both in alcoholic and nonalcoholic individuals. At least 200 instances of inadvertent hepatotoxicity have been recorded in heavy drinkers; they

have followed acetaminophen intake for 1 day to several weeks. In one series, 40% of individuals had taken acetaminophen in excess of 6 g/day (438), but 35% had taken doses below 4 g/day; in other reports, hepatotoxicity has occurred after as little as 1.5 to 2 g of acetaminophen intake a day. Data from the US Acute Liver Failure group revealed that 60% of persons who had taken an unintentional overdose were using an acetaminophen–narcotic combination. It has been suggested that addiction (and later, tolerance) to the narcotic component induces repetitive use that may not always be recalled or disclosed by the patient (436). Alcohol enhances CYP 2E1 activity, and accompanying malnutrition may contribute to GSH depletion. As in acute self-poisoning cases, AST and ALT levels are often 40- to 1,000-fold elevated above the ULN; this allows ready distinction of cases of acetaminophen hepatotoxicity from alcoholic hepatitis.

The importance of chronic excessive alcohol intake as a potentiator for susceptibility to acetaminophen hepatotoxicity has recently been challenged because in many case reports invoking such an interaction the person had clearly taken a hepatotoxic dose of acetaminophen (412). Nonetheless, the authors' experience is that therapeutic misadventures with acetaminophen are a common and clinically important type of drug-induced liver injury (434,446). A recent study of alcohol–acetaminophen interaction was conducted in an alcohol detoxification unit, with all trial participants receiving acetaminophen 1 g q.i.d. for 2 days or placebo (447). No differences in hepatotoxicity were observed between the two groups. However, the investigators' assertion that acetaminophen can be safely used in patients with chronic alcohol abuse has been criticized on several grounds, including the selection of only those persons with serum AT levels less than 120 U/L, which could have excluded a subset with significant underlying liver disease (448). The FDA recommends that persons taking more than three drinks of alcohol daily should not receive acetaminophen.

In nonalcoholic patients, fasting (449) has emerged as one of the most important risk factors, particularly after near complete deprivation of carbohydrate intake for at least 48 hours; this is a particularly important risk factor for acetaminophen hepatotoxicity in young children. Fasting decreases the activity of hepatic conjugation pathways for acetaminophen elimination, increases CYP 2E1 activity, and depletes hepatic GSH levels. However, others have contended that fasting has been overstated as a risk factor for acetaminophen hepatotoxicity (450). They contend that earlier studies were flawed because CYP 2E1 and GSH measurements had not been carried out simultaneously; depletion of GSH level is also accompanied by a decrease in CYP 2E1 activity (450).

Concurrent medication (e.g., INH, zidovudine, phenytoin, and other anticonvulsants) are also important. These agents compete for the "safe" conjugation pathways and may also induce CYP 3A4 (phenytoin) or 2E1 (INH), the net effect of which is to promote CYP-mediated oxidation of acetaminophen to its reactive intermediate, *N*-acetyl-*p*-quinone imine (NAPQI). However, human studies do not appear to support a phenytoin–acetaminophen adverse interaction. It is contended that phenytoin induces only CYP 3A4, a relatively minor metabolic pathway; further, it enhances

glucuronidation, a key detoxification step (450). Severe cardiopulmonary disease and renal failure have also been described as settings for acetaminophen hepatotoxicity (451), although the importance of metabolic factors or impaired (fluctuating) hepatic blood flow was not clarified in these studies.

Mechanism of liver injury and basis for antidote therapy

Acetaminophen undergoes metabolism to glucuronide and sulfate conjugates, which are excreted in the urine. Normally, only a small proportion (approximately 5%) of acetaminophen is oxidized to NAPQI; this reaction is catalyzed by CYP 2E1 (which increases with fasting, and INH and chronic alcohol intake) and, to a lesser extent, by CYP 3A4 (induced by anticonvulsants, several other drugs, and possibly alcohol). The small amounts of NAPQI formed with pharmacologic doses of acetaminophen are readily detoxified by hepatic GSH; hepatorenal injury occurs only when GSH reserves are depleted. This is the basis for the therapeutic rationale of administering thiol donors such as NAC and methionine, which replenish intracellular GSH stores (440). NAPQI binds cellular proteins, inducing oxidation of thiol groups in mitochondria, leading to mitochondrial permeability transition (452). The ensuing mitochondrial dysfunction generates profound oxidative stress and also facilitates peroxynitrate formation; the latter can also undergo covalent binding with key proteins and also further aggravate mitochondrial dysfunction. These events culminate in oncotic necrosis (oncosis) of hepatocytes and sinusoidal endothelial cells. Although apoptosis does occur, oncosis is considered the principal mode of cell death (453).

Depletion of natural killer (NK) cells and NK cell with T-cell (NKT cell) receptors appears to protect against acetaminophen liver toxicity in mice (454). The reduction in liver injury was accompanied by decreased expression of proinflammatory cytokines such as interferon-γ, key chemokines, and neutrophil recruitment. On this basis, it has been suggested that these cells, which are part of the hepatic innate immune system, may contribute to the progression of acetaminophen-induced liver injury (454).

Prevention

Adherence to the recommended therapeutic dose should be stressed. However, both prescribers and consumers should be made aware of the increased risk of acetaminophen toxicity in those who consume excess alcohol and in the setting of prolonged fasting, cardiorespiratory disease, and where other drugs are also being used. Recently, doubts have been expressed about the safety of acetaminophen in patients with underlying liver disease. Although acetaminophen is not contraindicated in such persons, the finding of detectable serum levels of acetaminophen in 20% of those with acute viral hepatitis–related acute liver failure has raised questions on the safety of acetaminophen in both acute and chronic liver disease (455). There is a

public health need to revise downward and promulgate altered dosage guidelines for acetaminophen when used in regular daily doses under any of the high-risk circumstances mentioned earlier. To reduce the impact of impulsive self-poisoning, attempts have been made to restrict pack sizes and change the packing (to bubble packs) of acetaminophen by legislation in the United Kingdom. This has been associated with a reduced number of liver transplantations for acetaminophen overdose (by approximately 66%), as well as a reduction in mortality (by 21%) (455). However, the impact has not been uniform, with only a transient decline in Scotland, and it remains unclear whether the decline in adverse outcomes is attributable to this measure alone (456).

Management

Gastric lavage with a wide-bore tube is performed in all patients presenting within 4 hours of acetaminophen overdose. Activated charcoal and osmotic cathartics are of no benefit. Serum levels of acetaminophen are often determined at baseline, but a 4-hour postingestion level is a more reliable predictor of the risk of liver injury (457). The need for antidote treatment is assessed using blood acetaminophen levels with reference to well-established nomograms (458,459). The 4-hour serum acetaminophen concentration may be misleading in overdoses with extended-release acetaminophen preparations (460); blood acetaminophen levels should be estimated after a further 4 to 6 hours (460). In persons presenting after nonaccidental or staggered ingestion of acetaminophen, the total dose ingested and time from ingestion to presentation are important determinants of liver injury. In these cases, hepatotoxicity is likely if the interval exceeds 24 hours or if the total acetaminophen dose exceeds 150 mg/kg (75 mg/kg in high-risk individuals); NAC therapy is initiated beyond these thresholds (461).

NAC is the principal antidote. By functioning as a thiol donor, NAC replenishes intracellular GSH stores. Significant hepatotoxicity is rare when NAC is administered within 16 hours of drug ingestion. Beyond 16 hours, oxidation of acetaminophen to NAPQI is complete and thiol donation is unlikely to prevent hepatocyte or renal tubular cell death. Nevertheless, the benefits of NAC have been shown extend to patients presenting up to 24 hours and even in those with acute liver failure (462). The beneficial effects of NAC in acute liver failure were attributed to improved tissue

oxygen delivery (462), although this has been disputed (463).

NAC is administered intravenously in Europe and Australia, and by mouth in the United States (459,464). The intravenous regime is now approved by the FDA for persons who cannot or will not use oral NAC.

Treatment protocol

An oral loading dose of 140 mg/kg is followed by 4-hour administration of half this dose up to 72 hours. Intravenous administration involves a loading dose (150 mg/kg) of NAC given slowly over 15 minutes, followed by a 4-hour infusion (50 mg/kg) and a 100-mg/kg infusion over 16 hours. A third intravenous (48-hour) protocol has also been evaluated (465). It has been suggested that the incidence of hepatotoxicity is lower with the oral and 48-hour intravenous regimes when compared to the 20-hour intravenous infusion among high-risk patients (460), but direct comparisons have not been performed. Only the 20-hour intravenous protocol has been approved by the FDA.

Anaphylactoid reactions to intravenous NAC are relatively common (6% to 15%). They are generally mild and rarely lead to treatment discontinuation, but severe reactions can occur. Guidelines to deal with such reactions (466) include observation during drug administration (with appropriate antidotes readily available), discontinuing the infusion with the onset of angioedema or respiratory symptoms, administration of antihistaminics, and resumption of the infusion after 1 hour if there are no persistent symptoms. For minor reactions, such as flushing, the infusion can be slowed or continued uninterrupted (466).

Other thiol donors such as methionine may be effective but must be administered within 10 hours. Methionine solutions should be freshly prepared, else they often cause troublesome vomiting; their use is restricted to patients with hypersensitivity to NAC. Managing acetaminophen overdoses in pregnant women and in children is along usual lines (460); in one small study, NAC did not have any major adverse effects on the fetus (467).

Treatment of acute liver failure is along usual lines (see Chapter 21). Assessment for liver transplantation requires consideration of the psychosocial factors underlying self-poisoning and the likelihood of survival without transplantation. When liver transplantation has been performed, survival rates exceed 70% (434) and long-term outcomes appear reasonable. However, deaths have occurred in survivors from repeat overdoses, stressing the need for detailed psychiatric evaluation and support before and after transplantation (468).

Dextropropoxyphene

Dextropropoxyphene is an opioid that is commonly used in compound analgesics, as well as on its own. Adverse drug reactions are rare but important, particularly because liver injury may occur in the postoperative period or in other medically complex situations. At least 25 cases of cholestasis with bile duct injury have been reported (39,84), some proved by inadvertent rechallenge. Most of those affected are women. Abdominal pain is the most impressive symptom. It is often severe and resembles pain from other causes of cholangitis with which it is often confused, particularly because jaundice is usually present. However, the large bile ducts appear normal at cholangiography. Liver histology shows portal tract edema, irregularity and necrosis of the biliary epithelium, bile ductular proliferation, and a peribiliary infiltrate of neutrophils and eosinophils.

Cholestasis is usually evident. Recovery occurs within 1 to 3 months of stopping dextropropoxyphene.

Nonsteroidal anti-inflammatory drugs

NSAIDs are among the most commonly used prescription and nonprescription drugs, with up to 15% of the population using them in any 1 year. AT abnormalities are observed in up to 15% of patients taking NSAIDs, but overt hepatotoxicity is much less common with currently used agents. There are distinct differences in the frequency of hepatic injury associated with individual NSAIDs (469,470), with some agents being virtually free from reported hepatotoxicity and others such as *bromfenac*, *benoxaprofen*, and *ibufenac* having been withdrawn because of fatal liver injury (471) (Table 33.23). Several pharmacoepidemiologic studies have attempted to categorize risk factors associated with NSAID-associated liver injury and to also ascertain the hepatotoxic potential of individual NSAIDs. The results of these studies have been conflicting, which could be attributed to methodologic differences, including study settings (hospital vs. ambulatory care) and study definitions. Focusing on studies with significant clinical events (i.e., hospitalization and death), Rubenstein and Laine conducted a systematic review and found a slight (but not significant) increase in the risk of liver injury for current as compared to former NSAID users (odds ratio 1.2 to 1.7, *P* = not significant) (472). Contrary to general belief, women and the elderly were not found to be at greater risk. However, in another study carried out in an ambulatory care setting in France, gender differences were striking; after adjustment for confounding factors, there was a significant association between liver injury and NSAID use in women (odds ratio = 6.49 [1.67 to 25.2]) (473). Other risk factors identified in the Rubenstein review were rheumatoid

arthritis (as compared with osteoarthritis) and use of nimesulide or sulindac.

Table 33.23. Liver Injury Associated with Salicylates and Nonsteroidal Anti-Inflammatory Drugs

Drug	Pattern of liver injury	Comments
Aspirin and other salicylates	Dose-dependent hepatocellular injury; Reye's syndrome (febrile children)	Similar toxicity with sodium salicylate, mesalamine; diflunisal causes cholestasis
Bromfenac	Acute hepatitis; fulminant hepatic failure	Withdrawn from clinical use
Cyclo-oxygenase 2 inhibitors	Cholestatic hepatitis (few reports) with celecoxib, rofecoxib	Overall incidence low
Clometacin	Acute or chronic hepatitis, cholestatic hepatitis, cirrhosis, granulomatous hepatitis	Fatalities recorded; female predominance (chronic hepatitis); some reported cases were later found to have hepatitis C virus infection
Diclofenac	Acute hepatitis; chronic hepatitis	Women, older patients with osteoarthritis
Ibuprofen	Acute hepatitis; rarely vanishing bile duct syndrome; hepatotoxic potential is low	Hepatocellular injury with pirprofen, fenoprofen, flurbiprofen, ketoprofen; cholestasis (tiaprofenic acid)
Indomethacin	Acute hepatitis, cholestasis; massive hepatic necrosis (rare)	Low incidence of toxicity
Naproxen	Mixed liver injury	Low incidence of toxicity
Nimesulide	Acute hepatitis; acute liver failure; cholestasis	Female predominance (with acute hepatitis)
Phenylbutazone	Acute hepatitis, cholestasis, granulomatous hepatitis	Similar toxicity as oxyphenbutazone
Piroxicam	Hepatocellular, cholestasis,	Low incidence; isoxicam, droxicam cause

	massive/submassive hepatic necrosis (at least six reports)	acute cholestatic hepatitis
Oxaprozin	Hepatocellular; fulminant hepatitis	—
Sulindac	Cholestatic hepatitis (predominant), acute hepatitis	Hypersensitivity features common

NSAID-associated hepatic disease encompasses a clinicopathologic spectrum from acute self-limited hepatitis, cholestasis, cholestatic hepatitis, and hepatic granulomas to fulminant hepatic failure, chronic hepatitis, and chronic cholestasis with ductopenia (471) (Table 33.23). Consistent with the diverse chemical structures of NSAIDs, both immunologic and metabolic idiosyncratic mechanisms have been invoked.

Sulindac

Sulindac is structurally related to indomethacin but has been implicated more often in liver injury. Cholestatic reactions predominate, although appreciable hepatic inflammation was noted in 25% of cases (473a). Sulindac has also been associated with acute pancreatitis, and this may cause extrahepatic biliary obstruction. Women appear more susceptible to liver injury (female-to-male ratio 3.5:1). Approximately 70% of affected individuals are older than 50 years (473a). Hypersensitivity features are common, as evidenced by the presence of fever, eosinophilia, and cutaneous reactions, including the Stevens-Johnson syndrome; this indicates operation of the RMS. Resolution often follows drug cessation but protracted cholestasis can occur. The overall case-fatality rate is approximately 5%, attributable both to sequelae of systemic hypersensitivity reactions (such as Stevens-Johnson, nephrotoxicity) and liver injury.

Ibuprofen

Ibuprofen rarely causes significant hepatic injury. In the few reported cases, the reactions have been hepatocellular or mixed hepatocellular–cholestatic (14). There are three case reports of VBDS, including two pediatric cases with associated Stevens-Johnson syndrome; two of these were referred for liver transplantation and the other had prolonged cholestasis (474,475). The presence of rash, fever, and eosinophilia suggests an immunoallergic basis. Rare cases of subacute liver failure have followed both overdose and therapeutic doses of ibuprofen. Explant histology showed submassive necrosis (474) or microvesicular steatosis (476). Recent reports of acute hepatitis induced by ibuprofen ingestion among HCV-positive patients are of interest but need to be confirmed by larger prospective studies (56).

Other propionic acid derivatives

Bromfenac was implicated in several cases of acute liver failure resulting in death or necessitating liver transplantation (477). In most cases, treatment had been provided for more than 90 days before the patients developed malaise and fatigue, followed by symptoms of severe hepatitis that progressed to liver failure over 5 to 37 days. There were no extrahepatic features of drug allergy. The liver pathology showed confluent or zonal necrosis and a predominantly lymphocyte infiltrate. Other propionic acid derivatives associated with

liver injury include *fenoprofen*, *ketoprofen*,*pirprofen*, and *tiaprofenic acid* (114) (Table 33.23).

Diclofenac

Diclofenac has been implicated in more than 200 published cases of hepatic injury (478,479,480), some severe and with occasional fatalities. In several cases, causality has been proved by inadvertent rechallenge. Significant hepatotoxicity occurs in approximately 1 to 5 per 100,000 persons exposed (23). Acute hepatitis or mixed hepatocellular–cholestatic injury is characteristic, but chronic hepatitis resembling AIH has been reported (481). Women, the elderly, and patients with osteoarthritis appear to be susceptible to diclofenac-induced liver injury. A comparative study of diclofenac with nabumetone found a better safety profile for the latter in elderly patients, with no patient developing ALT level elevation greater than 2 × ULN as compared to 4% in the diclofenac group (482). However, the relevance of ALT level elevation to cases of overt liver disease is unclear.

A prodromal illness or symptoms of hepatitis herald the onset of liver injury, most often within 3 months (range, 1 to 11 months) of starting diclofenac treatment. This is followed by jaundice and liver failure in severe cases. Fever and rash occur in 25% of cases. Liver tests usually reflect acute hepatitis, but some features of cholestasis may be present; jaundice occurs in 50% of reported cases (480). In some cases, the clinicopathologic features of ascites, hypoalbuminemia, and hyperglobulinemia indicate chronic liver disease. Liver biopsy specimens usually show acute lobular hepatitis, but confluent necrosis may be seen in severe cases; in chronic cases, periportal inflammation (interface hepatitis) and fibrous expansion of portal tracts are

noteworthy.

Resolution usually follows cessation of diclofenac, although fatalities have been recorded, particularly in elderly subjects. Corticosteroids were used successfully in a few cases of chronic diclofenac-induced hepatitis when no clinical improvement had been evident 3 months after drug discontinuation (479). Cross-sensitivity between NSAIDs is rare but has been reported with ibuprofen in a person with a history of diclofenac hepatitis; another had an adverse reaction to tiaprofenic acid (481).

Pathogenic mechanisms involved in diclofenac toxicity include oxidative stress alone or in combination with mitochondrial injury (482,483). In certain cases, immune mechanisms may be relevant, especially in those presenting with chronic hepatitis. The presence of diclofenac metabolite–protein adducts in liver tissue raises the possibility of liver injury resulting from direct disruption of critical cellular functions or from elicitation of an immune response to these neoantigens (484). Supporting this hypothesis is the finding that certain polymorphisms favoring a T helper 2 (T_H2)-mediated antibody response were found more often among patients with diclofenac hepatotoxicity than in healthy controls and persons receiving diclofenac without hepatotoxicity (485).

Piroxicam

The frequency of hepatic injury with this oxicam derivative is low (14). Acute hepatitis and cholestasis have been described, and there have been at least six cases of massive or submassive hepatic necrosis (14). The reaction appears to be dose independent, and immunoallergic features have not been conspicuous. Other oxicam derivatives implicated occasionally in cases of acute cholestatic hepatitis include *isoxicam* and *droxicam* (114).

Salicylates

Aspirin causes dose-dependent hepatic injury, usually with increased AT levels. Overt jaundice is uncommon, occurring in less than 5% of those affected (14). Patients with hypoalbuminemia, juvenile rheumatoid arthritis, and SLE are especially susceptible to salicylate-induced liver injury (14). Hepatitis resolves rapidly after drug withdrawal and usually does not recur after reintroduction of salicylates at a lower dose. Blood levels of salicylate should not exceed 24 mg/L (14). Only one fatality has been recorded (486). Focal necrosis and lobular inflammation are usual. Other salicylates can cause similar injury (487). Epidemiologic studies have linked the use of aspirin in febrile children with Reye's syndrome but concerted public health campaigns have led to a major decline (near abolition) in the incidence of this disorder (see Chapter 56).

Ticlopidine is not an NSAID but is discussed here with aspirin because it also inhibits platelet aggregation and is often used for similar indications. Unlike aspirin, ticlopidine-induced hepatotoxicity is dose independent and associated predominantly with cholestasis. More than 30 cases have been reported (82,488). Most cases have been found in individuals older than 55 years, reflecting the main use of ticlopidine as secondary prophylaxis against cerebrovascular and coronary artery disease. Onset of symptoms can be as early as 2 weeks, and in most cases is within 12 weeks of commencement. Rarely, symptoms may commence 1 month after the drug is withdrawn (489). Histology shows bland cholestasis, but cholestatic hepatitis with bile duct injury has been described. Recovery is usual within 3 to 6 months of stopping ticlopidine but occasionally may take longer than a year (490). Eosinophilia in some cases and a positive in vitro T-cell stimulation study to ticlopidine have been cited as favoring an immune basis for liver injury (491). Although corticosteroids

appear to have aided recovery in one patient (492), their routine use cannot be justified in this older group of patients (mean age, 67 years); one report of cytomegalovirus-associated acute hepatitis after corticosteroid therapy in this setting is a timely reminder of the risks associated with steroid use in this older group of patients (mean age, 67 years) (493). *Clopidogrel* has been successfully substituted for ticlopidine in a patient with cholestasis. However, clopidogrel can itself rarely cause hepatocellular or hepatocellular-cholestatic injury (494).

Cyclo-oxygenase 2 inhibitors

Celecoxib—in clinical trials, the frequency of hepatic dysfunction (0.8%) with this COX-2 inhibitor was not significantly different from that with placebo-treated (0.9%) subjects (495). However, celecoxib has been now incriminated in four reports of severe acute cholestatic hepatitis (114,496,497). Interestingly, two of these patients were allergic to sulfonamides; a history of sulfonamide allergy is considered by some to be an absolute contraindication to the use of celecoxib because there are structural similarities that predispose to hypersensitivity reactions. However, the implications of preexisting sulfonamide allergy are disputed by others (498), who contend that although celecoxib contains a sulfonamide moiety, it lacks the critical determinants that are implicated in sulfonamide allergy. One of the affected patients had alcoholic cirrhosis. This raises questions of celecoxib safety in persons with reduced hepatic reserve (497).

Symptoms suggestive of liver injury develop 4 days to up to 3 weeks after commencing celecoxib. Pruritus, jaundice, and malaise are accompanied by a mixed hepatocellular–cholestatic biochemical profile. Peripheral eosinophilia has been observed in some cases, suggesting a possible immunoallergic mechanism. Resolution occurs within 4 months of discontinuing the COX-2 inhibitor.

Rofecoxib, now withdrawn because of cardiovascular toxicity, was associated with a few reports of cholestatic hepatitis (114,499,500). Although the clinical features of liver injury resolved rapidly with drug withdrawal,

complete biochemical recovery could be delayed for as long as 2 years.

Nimesulide, another NSAID with preferential COX-2 selectivity, has also been associated with several instances of hepatocellular injury and cholestasis, and occasional cases of fulminant hepatic failure (501).

Allopurinol

Granulomatous hepatitis is a typical feature of liver injury with allopurinol (502). Other manifestations include increased AT levels in asymptomatic persons, cholestasis, and hepatocellular injury, which can be occasionally severe enough to progress to fulminant hepatic failure. Severe centrilobular hemorrhagic necrosis resembling Budd-Chiari syndrome has also been described (503). Exfoliative dermatitis, fever, eosinophilia, interstitial nephritis, and microangiopathic vasculitis may be present in these cases. In a few severe cases, corticosteroids have been used with apparent benefit (504). *Benzbromarone*, a uricosuric agent, has been implicated in causing chronic hepatitis, cirrhosis, and fulminant hepatic failure (505).

Gold

Hepatic toxicity of gold (gold sodium thiomalate) is usually characterized by mild cholestatic hepatitis. Onset is within 1 to 4 weeks of starting treatment. Fever, rash, and eosinophilia are often present. Liver biopsy specimens show canalicular cholestasis with minimal hepatocellular degeneration or portal tract inflammation. Resolution is the rule; fatalities are extremely rare (506). Rarely, prolonged cholestasis with ductopenia and other accompanying features of the VBDS (e.g., sialadenitis, sicca syndrome) can occur (507). Hypersensitivity features such as skin rash and peripheral and tissue eosinophilia were present in this case.

Hepatocellular injury is less common but can be severe, resulting in death (508,509). This severe reaction to gold appears to be dose dependent and is more likely the consequence of metabolic toxicity than in cases presenting with cholestatic hepatitis. Submassive or massive hepatic necrosis with a mixed inflammatory infiltrate is observed on liver biopsies. Resolution can be slow (510). Gold accumulates in the lysosomes ("aurosomes") in persons undergoing long-term crysotherapy. It has been proposed that liver injury occurs when the lysosomal storage capacity for gold is exceeded (510). Other gold compounds containing the same *aurothio* side group as gold sodium thiomalate (e.g., gold thiosulfate, aurothioglucose) are also associated with similar liver injury. Recently, acute cholestatic hepatitis has been reported after an overdose of *gold potassium cyanide*, a nonaurothio gold compound used in electroplating (511).

Penicillamine

Used in the treatment of Wilson disease and as a disease-modifying agent in rheumatoid arthritis, D-penicillamine has been associated with a range of side effects from nephrotoxicity to provoking autoimmune phenomena. Liver injury is less frequent. Most reports are of cholestatic hepatitis in association with hypersensitivity features (512). These occur within 2 weeks of commencing treatment. The prognosis is good, with resolution occurring within few weeks of cessation of penicillamine; fatalities are rare (513).

Leflunomide

Leflunomide is a disease-modifying antirheumatic drug. Its principal metabolite, A771726, is highly metabolized and eliminated through the liver. CYP 2C9 is probably involved in its metabolism. In clinical trials, 5% of recipients showed mild, reversible changes in AT. Concerns about significant liver toxicity were first highlighted by the European Medicines Evaluation Agency, which had received 296 reports of hepatic adverse effects, including 15 cases of liver failure; 9 of these cases had a fatal outcome. However, assigning a cause–effect relationship has proved problematic because many (78%) were receiving potential hepatotoxic drugs, including NSAIDs and methotrexate. Furthermore, other confounding factors were present such as alcohol use, abnormal baseline liver test results, congestive heart failure, and failure to comply with recommended doses. The low hepatotoxic potential of leflunomide compared with methotrexate was confirmed in two large cohorts involving over 40,000 patients (514). A special committee of the FDA concluded that the risk of hepatotoxicity was small with leflunomide, thereby permitting its continued usage (515).

Special caution is advised when leflunomide is used with methotrexate. AT level increase is seen more often in persons receiving this combination (22%) as compared to recipients of methotrexate plus placebo (5%). However, normalization of AT levels was achieved without dose change (59%) or a single dose decrease (29%) (516). Nevertheless, continued vigilance is necessary because early changes of cirrhosis were observed in a patient taking both these drugs (516a). It has also been suggested that persons carrying a low–metabolizing activity polymorphism (e.g., CYP 2C9*3) may be at an increased risk of liver injury (517).

The manufacturer recommends baseline and monthly monitoring of liver test results for the first 6 months, and every 2 months thereafter. Minor ALT level changes (<2 × ULN) should prompt retesting in 2 to 4 weeks. Dose reduction (from 20 to 10 mg) is suggested if the ALT level increases above 2 × ULN. Increases of ALT levels greater than 3 × ULN or persistent ALT abnormalities should prompt drug withdrawal, together with the use of cholestyramine to facilitate the elimination of leflunomide.

Oncotherapeutic and Immunosuppressive Drugs

Systemic malignancy is commonly associated with abnormal liver test results (14). In addition to the direct and indirect effects of neoplastic cells, radiotherapy, sepsis, concurrent medication, viral hepatitis, parenteral

nutrition, and hypoxemia may contribute to hepatic liver test result abnormalities; this makes it difficult to interpret changes or assign liver toxicity to individual drugs. An emerging problem is the reactivation of quiescent chronic hepatitis B after withdrawal from the immunosuppressive effects of chemotherapy; this can be prevented by preemptive lamivudine therapy (see Chapter 29). Interactive toxicity between individual oncotherapeutic drugs also merits special consideration. This is exemplified by the development of hepatic SOS with combination chemotherapy and the potentiation of doxorubicin hepatotoxicity by 6-mercaptopurine.

Antimetabolites and antibiotics are more often linked to significant liver injury than are alkylating agents. Hepatocellular injury is characteristic, but a wide range of histologic lesions may be encountered (14,518). Steatosis is often present, especially with dactinomycin, L-asparaginase, and methotrexate. Cholestasis is typically associated with the hormonal agents but can be a feature of liver injury with azathioprine and IL-2. A range of vascular lesions, including peliosis hepatis, SOS (VOD) and NRH, has been observed, usually in the setting of bone marrow or renal transplantation. Chronic hepatitis can rarely occur with doxorubicin and azathioprine, but cirrhosis is distinctly uncommon, except with methotrexate (discussed subsequently). The hepatotoxicity of commonly used oncotherapeutic agents is summarized in Table 33.24. More in-depth discussions of the subject can be obtained from recent reviews and texts (2,14,518).

Methotrexate

The hepatotoxic potential of methotrexate was recognized soon after it was introduced in the 1950s for the treatment of acute childhood leukemia; significant hepatic fibrosis and cirrhosis were reported in up to 25% of patients (14). Methotrexate is now used more often in a low-dose weekly regime for treating rheumatoid arthritis, psoriasis, and other immunologic conditions, including inflammatory bowel disease. The hepatoxicity of low-dose regimens is much lower but is still debated (29,519,520); although ultrastructural changes can occur, clinically significant liver disease is now rarely, if ever, encountered.

The development of hepatic fibrosis during methotrexate therapy is greatly influenced by the factors listed in Table 33.25. Pretreatment variables, such as older age, renal failure, preexisting liver disease, and risk factors for NASH (e.g., diabetes, obesity) should be carefully considered before commencing treatment with methotrexate (519). Alcohol intake, preexisting liver disease, and the total and incremental dose are the most important of these risk factors. In earlier studies, a cumulative methotrexate dose of 3 g was associated with histologic progression in 20% of cases, but only 3% had advanced fibrotic changes (29). In a

meta-analysis of 15 studies, moderate or heavy drinkers (>100 g/week) were more likely to have advanced histologic changes (18%) and show histologic progression (73%) (29). Compared to those with rheumatoid arthritis, patients with psoriasis were also more likely to have advanced changes (7.7% vs. 2.7%) and histologic progression (33% vs. 24%). Finally, it is noteworthy that preexisting liver test result abnormalities are observed in 25% to 50% of patients with psoriasis and rheumatoid arthritis.

Table 33.24. Liver Injury Associated with Oncotherapeutic Drugs

Drug	Pattern of liver injury
ANTIMETABOLITES	
Azathioprine	Cholestasis; vascular injury–peliosis hepatis, SOS (VOD)
Cytosine arabinoside	Raised AT level (with low dose), cholestasis or hepatocellular injury (high doses)
5-Fluorouracil	Rare liver injury with intravenous and intra-arterial route (particularly when associated with levamisole); floxuridine causes primary sclerosing cholangitis–like lesions
Gemcitabine	Transient AT level changes; cholestatic hepatitis leading to acute liver failure; SOS
6-Mercaptopurine	Bland cholestasis, hepatocellular or mixed injury; rarely fatal hepatic necrosis
Methotrexate	Steatosis, hepatic fibrosis, cirrhosis
6-Thioguanine	VOD (in combination); peliosis; hepatocellular injury or cholestasis (rare)

ANTIBIOTICS	
Bleomycin	Low incidence of liver toxicity
Cyclosporine	Mild cholestasis (more accurately—impaired bilirubin transport), usually reversible with dose modification
Doxorubicin	Rarely acute or chronic hepatitis; hepatotoxicity enhanced by 6-mercaptopurine
Dactinomycin	Hepatopathy–thrombocytopenia syndrome (with vincristine); clinically resembles SOS (VOD)
Daunorubicin	SOS (when used in combination treatment)
Mithramycin (plicamycin)	Raised AT level (in up to 100%); occasionally centrilobular necrosis, steatosis
Mitomycin C	SOS, steatosis
Mitoxantrone	Minor increase in AT level
SPINDLE INHIBITORS	
Vincristine	Transient liver enzyme changes; synergistic with irradiation in producing liver injury
Paclitaxel, docetaxel	Minor increase in AT level
PLATINUM	Cisplatin
Liver injury rare	Raised AT level (high doses); steatosis, cholestasis, minor hepatic necrosis
Carboplatin	Acute liver failure (one case), SOS (with etoposide)
TOPOISOMERASE INHIBITORS	
Etoposide Irinotecan, topotecan	Frequent liver test abnormalities (high dose); occasionally severe hepatitis (standard dose) Abnormal liver tests
ALKYLATING AGENTS	Low incidence of liver injury with this group
Busulfan	Cholestasis, porphyria cutanea tarda, nodular regenerative hyperplasia, SOS (in combination)
Capecitabine	Hepatocellular
Chlorambucil	Hepatocellular, cholestatic hepatitis, acute liver failure
Cyclophosphamide	Hepatocellular, SOS (in combination), steatosis

Ifosfamide	Liver injury rare; cholestasis (two cases)
Melphalan	Minor increase in AT levels
Thiotepa	Severe hepatotoxicity resembling phosphorus poisoning (rare)
NITROSOUREAS	
BCNU, CCNU, streptozotocin	Minor increase in AT level; rare fatalities from liver injury (BCNU, CCNU)
HORMONAL AGENTS	
Aminoglutethamide	Cholestasis
Flutamide	Cholestatic hepatitis, fulminant hepatic failure; megestrol acetate associated with cholestasis
Tamoxifen	Steatosis, nonalcoholic steatohepatitis, rarely submassive hepatic necrosis, and cirrhosis
MISCELLANEOUS DRUGS	
Amsacrine	Hepatocellular injury, cholestasis, rarely fatal hepatic necrosis
L-Asparaginase	Microvesicular steatosis, hepatic necrosis, coagulopathy (also with pegasparaginase)
Dacarbazine	SOS (VOD)
Procarbazine, hydroxyurea	Hepatocellular injury

SOS, sinusoidal obstruction syndrome; VOD, veno-occlusive disease; AT, aminotransferases; BCNU, carmustine; CCNU, lomustine.
Original references can be obtained from refs. 2, 14, and 518.

Table 33.25. Risk Factors for Methotrexate Liver Toxicity

Risk factor	Importance	Implications for prevention
Age >60	Increased risk (reduced renal clearance may contribute)	Greater care in use of methotrexate for older people
Dose	Incremental dose Dose frequency Duration of therapy Cumulative (total) dose	5–15 mg/wk very safe Weekly bolus (pulse) safer than daily schedules Consider review of hepatic status every 2 to 3 y Review hepatic status after every 2 g methotrexate
Alcohol	Increased risk with daily levels	Avoid methotrexate use if intake not curbed

consumption	of >15 g (one to two standard drinks)	Consider pretreatment liver biopsy
Obesity, diabetes, metabolic syndrome	Increased risk	Consider pretreatment and progress liver biopsies
Preexisting liver disease	Greatly increased risk Particularly related to alcohol, obesity, and diabetes (nonalcoholic steatohepatitis)	Pretreatment liver biopsy mandatory Avoid methotrexate, or schedule progress biopsies according to severity of hepatic fibrosis, total dose, and duration of methotrexate therapy Monitor liver test results during therapy (see text)
Folate supplements	Increased risk of liver injury in persons not receiving folic acid	Concurrent folate therapy recommended
Systemic disease	Risk greater with psoriasis than rheumatoid arthritis	None
Impaired renal function	Reduced systemic clearance of methotrexate	Reduce dose; greater caution with use

Clinical features and laboratory data

Minor increases in ALT levels are seen 1 to 2 days after starting methotrexate treatment. These changes bear little relevance to the development of hepatic fibrosis, which can only be assessed by liver biopsy. Clinical features are absent or nonspecific for liver disease until complications of portal hypertension and liver failure develop. In these now rare advanced cases, hepatosplenomegaly, ascites, muscle wasting, thrombocytopenia, and hypoalbuminemia can be noted, but jaundice, hyperbilirubinemia, and coagulation disturbances are distinctly uncommon.

Histology

The specific scoring system, devised by Roenigk et al. is widely used for grading liver histology in methotrexate users (521). In this system, grades I and II indicate a variable amount of steatosis, nuclear pleomorphism, and necroinflammatory activity but fibrosis is absent. Higher grades reflect increasing degrees of fibrosis, as follows: Grade IIIA (few septa), grade IIIB (bridging fibrosis), and grade IV (cirrhosis). The pattern of hepatic fibrosis includes striking pericellular fibrosis, a feature of both alcoholic steatohepatitis and NASH; the possibility that methotrexate itself causes steatohepatitis or accentuates fibrogenesis among persons with underlying “primary NASH” has been suggested (52). However, cases have been reported in which hepatic fibrosis appeared in livers with a relative paucity (or complete absence) of portal and lobular inflammation. Because the extent of hepatic fibrosis is the only important abnormality in those taking methotrexate, Richards et al. have proposed a new semiquantitative method for the evaluation of liver biopsy specimens in patients with rheumatoid arthritis (522). The objective is to provide greater sensitivity for detecting early hepatic fibrosis than the Roenigk system, and further validation will be of interest.

Prevention of methotrexate fibrosis

Coprescription of folic acid is associated with a lower risk of liver injury (odds ratio [confidence interval (CI)] 0.10 [0.04 to 0.21]) (523) without sacrificing efficacy. However, its impact on preventing hepatic fibrosis is unknown (524). Guidelines have been published for monitoring methotrexate therapy (519,525,526,527) in patients with rheumatoid arthritis and psoriasis (summarized in Table 33.26). Less stringent guidelines for monitoring have been suggested. Yazici et al. have proposed less frequent liver tests (every 3 to 4 months), without additional risks but with reduced costs to the patient (528). However, the generalizability of these suggestions has been questioned because they have been based on a study population that was younger than that reported in other series and the patients were receiving lower doses of methotrexate (529).

A revised threshold for liver biopsy has also been proposed (530). In this British retrospective study, advanced liver fibrosis was found in 2.6% and 8.2% of patients with psoriasis who had received a cumulative dose of 4 and 5 g, respectively. On this basis, liver biopsy was recommended after a cumulative methotrexate dose of 5 g (530). The value of liver biopsies, to

assess methotrexate hepatotoxicity in diseases other than psoriasis and rheumatoid arthritis, has not been established. In a study of 32 patients with inflammatory bowel disease receiving long-term methotrexate (mean dose 2.6 g; follow-up 131 weeks), histologic changes were common but minor, and significant hepatic fibrosis was rare (531). Similar considerations apply to methotrexate use in sarcoidosis; hepatic reactions were common, but it proved difficult to separate drug toxicity from sarcoidosis-related liver features (532).

Table 33.26. Guidelines for Monitoring Hepatotoxicity in Patients with Rheumatoid Arthritis and Psoriasis During Methotrexate Treatment

Approach	Psoriasis (Said 1997, Roenigk 1998)	Rheumatoid arthritis (Kremer 1994)
Pretreatment (laboratory tests)	Routine liver tests, full blood count, urinalysis, blood urea, creatinine, creatinine clearance, hepatitis B/C serology	Same as psoriasis
Pretreatment (liver biopsy)	Not mandatory	
	Early treatment biopsy (2 to 4 m) in patients with risk factors for liver disease (past/current ethanol intake greater than one to two drinks/d, abnormal liver test results, familial liver disease, diabetes, obesity, exposure to hepatotoxic drugs)	Recommended in patients with history of excessive ethanol intake, abnormal baseline AST values, chronic hepatitis B or C virus infection[a]
During treatment (laboratory tests)	Liver tests 4 to 8 wk (1 wk after last dose). Frequent monitoring during initial treatment, dose escalation during episodes in which blood MTX levels could be elevated (dehydration, impaired renal function, nonsteroidal anti-inflammatory drug use)	Recommended in patients with history of excessive ethanol intake, abnormal baseline AST, chronic hepatitis B or C virus infection
	With significant and persistent abnormalities, withhold MTX 1 to 2 wk and repeat tests If abnormalities persist, consider liver biopsy	
During treatment (liver biopsy)	This is with no risk factors for liver disease, and normal physical examination and liver tests: Liver biopsy recommended after cumulative MTX dose 1.5 g[b]; if biopsy is normal, repeat at subsequent 1 to 1.5 g cumulative dose[c]	Liver biopsy recommended if five of nine AST determinations within 12 m (6 of 12 if tests performed monthly) are above upper limit of normal or serum albumin level falls (less than normal) in controlled rheumatoid arthritis

[a]The authors agree that this should now be 2 to 4 g.
[b]Additional 2 g cumulative dose (modified by earlier biopsy findings).
[c]Current understanding would add "risk factors for nonalcoholic fatty acid liver disease/nonalcoholic steatohepatitis, especially metabolic syndrome."
MTX, methotrexate; AST, aspartate aminotransferase.

Noninvasive methods of assessing hepatic fibrosis have been proposed for use during methotrexate therapy but have yet to be fully validated and adopted. These include measurement of serum procollagen-III peptide (PIIIP) levels (533) and dynamic hepatic scintigraphy. Doubts have been expressed about the reliability of PIIIP measurements in individual patients (534). Further, serial measurements are necessary to assess a dynamic process such as fibrogenesis (533). Dynamic hepatic scintigraphy evaluates the contribution of portal blood flow to the hepatic blood supply. Its application is based on the premise that changes in portal blood flow would be sensitive to structural alterations (534); a portal blood flow contribution of greater than 52% correlated with a 95% likelihood of normal liver histology.

Azathioprine

Azathioprine, the prodrug of 6-mercaptopurine, is used as an immunosuppressive agent or as a steroid-sparing agent in autoimmune diseases (see Chapter 31). Hepatic complications of azathioprine are rare but severe, diverse, and often of very late onset; all these factors provide a challenge for appropriate diagnosis and management. Disorders associated with azathioprine include bland cholestasis, cholestatic hepatitis with bile duct injury (535), zonal necrosis, and vascular toxicity (536,537). The latter encompasses diverse syndromes of SOS (VOD), peliosis hepatis, NRH, and noncirrhotic portal hypertension (537). HCC has also been recorded in a long-term recipient (538). Indirect hepatic effects of azathioprine such as opportunistic infections (e.g., cytomegalovirus) or, rarely, liver infiltration from lymphoma should always be considered.

6-Mercaptopurine causes dose-dependent hepatocellular necrosis, which can be fatal. Rarely, it is associated with cholestasis (14,539). Measuring 6-mercaptopurine metabolite levels (6-methylmercaptopurine) may be useful in making a diagnosis of drug hepatotoxicity in complex settings such as post–liver transplantation (539).

6-Thioguanine

Vascular toxicity, particularly hepatic SOS (VOD), is a characteristic feature of 6-thioguanine (6-TG) hepatotoxicity (Table 33.24) in the context of hematologic malignancies. Less well recognized is the development of NRH in persons with inflammatory bowel disease. In one study conducted in Los Angeles, 111 patients with inflammatory bowel disease receiving 6-TG were examined for potential hepatotoxic effects. Increase in liver enzyme levels and reduced platelet counts were found in 26% (540). These were seen more often in men and in those with preferential 6-methylmercaptopurine production during treatment with 6-mercaptopurine or azathioprine. Liver histology was available in 38 cases. Reticulin-stained sections showed NRH in 20 (53%); conventional hematoxylin–eosin sections identified NRH in only 4 of these cases. Ultrastructural studies demonstrated sinusoidal collagen deposition in 60% (14 of 23), a figure significantly higher than that observed with conventional trichrome stains (34%). SOS (VOD) was observed in one case (541). NRH was present more often (76%) in patients with abnormal laboratory tests than among those without such abnormalities (33%).

Cyclosporin A

Between 6% and 86% of organ transplant recipients receiving cyclosporine develop abnormal liver test results (14,542). However, these changes are mild and self-limiting, consisting of transient increase in SAP level, accompanied by a slight elevation in bilirubin and AT levels. Most patients do not develop symptoms of cholestasis or hepatitis. The biochemical changes are most evident in the first month after transplantation. However, up to 32% of cases may be associated with prolonged liver test abnormalities (542). Long-term recipients of cyclosporine are also at risk of developing biliary calculi (543), the consequence of altered bile flow and biliary lipid composition. Cyclosporine-induced liver test result abnormalities usually settle with dose reduction or discontinuation of treatment. There is anecdotal evidence that ursodeoxycholic acid may be beneficial in this setting (72).

In experimental models, cyclosporine induces cholestasis by inhibiting Bsep-mediated taurocholate transport, culminating in decreased bile flow (544). It also disturbs bile salt kinetics by inhibiting bile salt synthesis, reducing the size of the bile salt pool, and increases cholesterol saturation in bile by reducing phospholipid secretion (see Chapter on cholestasis for details).

Sirolimus

Increases in AT levels have been observed in liver and renal transplant recipients on sirolimus (545). Such cases were initially misdiagnosed as representing allograft rejection and were managed (unsuccessfully) with dose escalation. Resolution occurred only when sirolimus was withdrawn. Liver biopsy specimens show sinusoidal congestion or mild hepatitis.

Anticonvulsants

Phenytoin

Raised GGTP and SAP levels are very often observed in the absence of hepatic injury among people taking phenytoin; this usually reflects enhanced hepatic enzyme synthesis (a form of hepatic adaptation), but in those without adequate sunlight exposure, it is important to exclude vitamin D deficiency arising from enhanced hepatic metabolism of this vitamin. Acute hepatitis, including severe cholestatic hepatitis leading to VBDS, is a very rare but important side effect of phenytoin (2,546,547), usually as part of the RMS (Table 33.5). There may be an increased rate of hepatic reactions among African Americans compared with whites (546). Onset is usually within 6 weeks. Biochemical features reflect hepatocellular necrosis with high AT levels, but mixed patterns may occur. In phenytoin hepatitis, liver biopsy specimens show diffuse hepatocellular degeneration and multiple acidophilic (apoptotic) bodies, bridging necrosis, and a prominent lymphocytic or mixed cell inflammatory infiltrate containing neutrophils and eosinophils. Hepatic granulomas may also be present. The combined appearances of lymphocyte beading, mitotic hepatocytes, and granulomas resemble those observed in infectious mononucleosis (2).

Phenytoin was one of the first drugs associated with hypersensitivity features now regarded as characteristic of the RMS (anticonvulsant hypersensitivity syndrome or "pseudomononucleosis" syndrome) (30). Fever, severe forms of rash, lymphadenopathy, leukocytosis, and Stevens-Johnson syndrome are frequent, and the key link is with visceral involvement including the liver, kidney, bone marrow, and lung (Table 33.5). Resolution occurs with phenytoin withdrawal, but the case-fatality rate of patients developing liver injury is up to 20%. Continued ingestion of phenytoin after the onset of symptoms is associated with a poor outcome. Treatment consists of supportive measures. Corticosteroids have not proved beneficial.

Although the presence of hypersensitivity features suggests immunologic idiosyncrasy, it now seems likely that the primary abnormality is related to the generation of a highly reactive arene oxide metabolite. If detoxification of this metabolite by epoxide hydrolase is inadequate, it binds to cellular macromolecules

or initiates oxidative stress, causing cell injury with apoptosis or necrosis. The way in which this reactive metabolite incites profound drug hypersensitivity is less clear, but neoantigenic determinants of immune reactivity, cytokine mobilization, and defects in cell defenses against oxidative stress and proinflammatory stressors are potential candidates. A genetic deficiency of this enzyme has been identified in patients and their family members (548). CYP 2C9 is a key enzyme in phenytoin metabolism. Phenytoin-associated VBDS has been described in a patient heterozygous for CYP 2C9*3 (an allele conferring low enzyme activity); this supports a pharmacogenetic basis for this adverse drug reaction (549).

Carbamazepine

Liver disorders comprise approximately 10% of adverse drug reactions with carbamazepine (14). The frequency of liver injury is estimated at 16 cases per 100,000 treatment-years (14). Granulomatous hepatitis with varying degrees of hepatocellular injury and cholestasis have been reported (550,551,552,553,554). Children may also be affected, including neonates of women receiving carbamazepine during pregnancy (555). Onset is within 6 to 8 weeks. The hallmarks of hypersensitivity (e.g., fever, rash, angioedema, eosinophilia, and raised immunoglobulin E [IgE] levels) are often observed, linking this reaction as part of the RMS observed with other aromatic anticonvulsants (e.g., phenytoin, phenobarbitone) metabolized through arene oxides.

Submassive or massive hepatic necrosis has been noted in a few patients. Multiple hepatocellular adenomas were described in a man receiving carbamazepine for 17 years (556), but there is no evident biologic basis for an etiopathogenic role of the drug in this syndrome.

The case-fatality rate of carbamazepine hepatitis is 10% among those with hepatocellular reactions. Some reported cases of fulminant liver failure were also associated with concurrent acetaminophen intake or antituberculous therapy (557); the role of interactive hepatotoxicity needs to be considered in such severe cases. Patients presenting with granulomatous and/or cholestatic reactions usually survive, although bile duct injury and VBDS may rarely occur (88).

Lamotrigine

Indications for lamotrigine include partial and generalized seizures and as adjunctive therapy in children with refractory epilepsy. Early reports of liver test abnormalities were wrongly attributed to status epilepticus. Subsequently, a small but definite risk of hepatotoxicity was documented (558). However, attributing causality can be difficult because other potentially hepatotoxic antiepileptic drugs are often coprescribed.

Acute hepatitis was reported in ten persons, including two instances of fulminant hepatic failure. Symptoms developed within 2 to 3 weeks (range, 6 to 39 days). Extrahepatic features such as rash, disseminated intravascular coagulopathy, and rhabdomyolysis were sometimes present. Liver biopsy specimens showed acute hepatic necrosis or focal hepatitis with mild portal inflammation (558). Progressive hepatic necrosis culminating in fatal acute liver failure has been documented. In most other reported reactions, liver injury settled within a few weeks of stopping lamotrigine. Lamotrigine is structurally different from the aromatic anticonvulsants (e.g., phenytoin, phenobarbital, carbamazepine). Although in vitro cross-reactivity has been reported in patients with a history of reactions to the older antiepileptic drugs (559), there is no documented clinical cross-reactivity between lamotrigine and these agents.

Valproic acid (sodium valproate)

Risk factors

Up to 40% of recipients of VPA develop reversible increases in AT levels. These changes are frequently observed within in the first 2 months of treatment and are unrelated to the rare severe form of liver injury. VPA-associated hepatic injury is independent of dose and duration of treatment. It occurs predominantly in children, particularly those younger than 3 years. Among 37 fatal cases noted in a retrospective analysis of 400,000 persons taking VPA between 1978 and 1984, children younger than 10 years represented 73% (27 of 37 affected) (560). Risk factors include a family history of mitochondrial enzyme deficiencies (including urea cycle, long-chain fatty acid metabolism defects), Friedreich's ataxia, Reye's syndrome, having a sibling affected by VPA hepatotoxicity, and multiple drug therapy. There are 26 published cases of VPA liver toxicity in adults (561). The overall risk of liver injury among persons taking VPA is between 1 per 500 persons exposed among high-risk groups and less than 1 in 37,000 in low-risk groups (562).

Onset, clinical features, and laboratory findings

Symptoms begin 4 to 12 weeks after starting VPA and are often nonspecific; lethargy, malaise, poor feeding, somnolence, worsening seizures, muscle weakness, and facial swelling are important new symptoms among children prescribed VPA. They may be followed by features more readily attributable to hepatotoxicity, including anorexia, nausea, vomiting, weight loss, right upper quadrant discomfort, or abdominal pain. Jaundice, hypoglycemia, ascites, coagulation disorders, and encephalopathy indicate liver failure with imminent

coma and death. Another presentation is associated with fever and tender hepatomegaly suggestive of Reye's syndrome. The prognosis is better in such cases. Yet others exhibit prominent neurologic features, such as ataxia and confusion with little evidence of hepatic involvement. Additional extrahepatic features observed among patients with VPA hepatotoxicity include thrombocytopenia, pancreatitis, and alopecia. The biochemical features resemble those described earlier for other mitochondrial hepatotoxins; a modest rise in bilirubin and ALT level is accompanied by hypoalbuminemia, hyperammonemia, and profound impairment in serum levels of clotting factors synthesized by the liver.

Pathology

Histologic appearances include submassive or massive hepatic necrosis in two thirds of cases (562) and zonal or diffuse microvesicular steatosis in the others; steatosis may also accompany hepatic necrosis. Bile duct injury has been observed in a few cases, but as part of massive or submassive necrosis in which any additional significance is questionable.

Management, outcome, prevention

Management, outcome, and prevention include supportive measures for underlying metabolic defects and managing acute liver failure. In one retrospective study, L-carnitine supplementation reduced mortality (563); 20 (48%) of 42 patients treated with L-carnitine survived, as against only 5 (10%) of 50 patients managed by aggressive supportive care alone. The place of liver transplantation is unclear; it has been performed successfully in some cases (564), but in others it led to worsening of neurologic disease (565).

At least 60 deaths from VPA hepatotoxicity are recorded and the mortality remains high. Prevention is possible by adherence to prescribing guidelines; these include avoiding VPA in combination with other drugs in the first 3 years of life and in children with mitochondrial enzyme defects. Monitoring liver tests is unhelpful because of the high frequency of nonspecific liver test abnormalities. Children and their parents should be urged to report any new symptoms developing within the first 6 months of VPA therapy.

Mechanism of liver injury

Unlike the aromatic anticonvulsants, VPA hepatotoxicity is rarely accompanied by manifestations of the RMS (566). Metabolic idiosyncrasy is probably the principal mechanism of VPA toxicity. Glucuronidation and β-oxidation are the principal pathways of VPA metabolism, but VPA may be metabolized by CYP enzymes to 4-en-VPA, a pathway that is accentuated in persons taking concurrent medications (particularly other anticonvulsants) that induce CYPs. Some VPA oxy-metabolites, and notably 4-en-VPA, inhibit mitochondrial fatty acid β-oxidation. Other metabolic effects of VPA therapy include secondary carnitine deficiency, therapeutic correction of which may be valuable (see preceding text).

Topiramate

A woman receiving topiramate and carbamazepine developed acute liver failure. The explanted liver showed centrilobular necrosis (567). Another person prescribed topiramate for a bipolar affective disorder developed raised ALT level (>700 U/L), hypoalbuminemia, and mild hyperammonemia. Resolution occurred after topiramate was discontinued. Other medications including VPA could be resumed without ill effects (568). By contrast, topiramate used as an add-on anticonvulsant induced acute liver failure (569), which resolved only after VPA was withdrawn.

Felbamate

Cases of acute hepatitis and fatal fulminant hepatic failure have been attributed to felbamate (570). Most affected individuals were women. The overall frequency of liver injury and liver-related death was estimated at 1 per 7,000 and 1 per 125,000 persons exposed, respectively. Half the reported cases occurred between 3 and 6 months (range 2 weeks to 8 months). Generation of atropaldehyde, a reactive metabolite, could be critical to felbamate toxicity (571). Felbamate should be reserved for treating refractory epilepsy, especially the Lennox-Gastaut syndrome.

Gabapentin

Gabapentin has been implicated in causing cholestatic hepatitis (572). Another report of liver injury attributed to gabapentin has been disputed because other potentially hepatotoxic medicines had been coprescribed (573). The Committee on Safety of Medicines, the United Kingdom, has received reports of four other unpublished cases of jaundice, including one other cholestatic drug reaction. Overall, this drug has low hepatotoxic potential.

Antipsychotic Agents, Sedative-Hypnotics, and Antidepressant Drugs

Chlorpromazine

Chlorpromazine is historically one of the most important causes of drug-induced liver disease, both because of the relatively high frequency of idiosyncratic reactions and because it is the archetypical example of a drug causing cholestatic hepatitis; VBDS and other

complications, such as massive hepatic necrosis, also occur occasionally. Full descriptions of chlorpromazine hepatitis may be found elsewhere (2,14,574), but the essential features are recapitulated here because of what chlorpromazine has taught us about hepatic drug reactions and because the agent is still used occasionally.

Chlorpromazine is associated with cholestatic hepatitis in 0.2% to 2% of recipients; the risk increases with age and is higher in women. The onset of prodromal symptoms occurs within 1 to 6 weeks (574). Fever and nonspecific systemic complaints are present in over half the patients, but rash is uncommon. Jaundice, pruritus, and generalized or right upper quadrant pain occur later. SAP level is elevated more than threefold, along with a moderate rise in the AT level and variable increase in serum bilirubin level, depending on severity. Peripheral blood eosinophilia is present in 10% to 40% of those affected. Liver histology is characterized by centrilobular cholestasis, portal inflammation, mild parenchymal injury, and occasionally bile duct damage (574). Resolution occurs within 12 weeks in most cases, but approximately 7% develop VBDS.

The brisk response to rechallenge suggests immunoallergic idiosyncrasy but there is evidence of a toxic component to liver injury. Chlorpromazine and, particularly, its hydroxylated metabolites inhibit plasma membrane Na^+/K^+-ATPase, alter membrane fluidity, and polymerize actin. Genetically determined defects in sulfoxidation of chlorpromazine (the sulfoxide metabolite is inert) have been postulated but are unproved (575).

A similar pattern of cholestatic liver injury has been described less frequently with *prochlorperazine* (576) and other neuroleptics such as *haloperidol* (577), *pimozide*, and *sulpiride*, including rare cases of VBDS (82).

Sedative-hypnotics

Liver injury is extremely rare with the benzodiazepines and other minor tranquilizers, anxiolytics, or hypnotics. Both hepatocellular and cholestatic reactions are described, but often from times preceding the accurate diagnosis of all forms of viral hepatitis (14).

Antidepressants

Monoamine oxidase inhibitors

Iproniazid was one of the first drugs associated with acute hepatitis (2). These reactions occurred in 1% of recipients and were often severe, including instances of fatal fulminant liver failure. The hydrazine substituent (which iproniazid partly shares with INH, ethionamide, pyrazinamide, and nicotinamide) was probably the hepatotoxic moiety (578). Hydrazine sulfate can cause severe hepatorenal toxicity (578). *Phenelzine* and *isocarboxazid* have also been associated with occasional instances of hepatocellular injury, but monoamine oxidase inhibitors (MAOIs) are now rarely prescribed.

Tricyclic antidepressants

Tricyclic antidepressants bear structural resemblance to the phenothiazines and are an occasional cause of cholestatic or, less commonly, hepatocellular injury. Recovery after drug cessation is usual, but prolonged cholestasis has been observed with amitriptyline (579) and imipramine (580).

Selective serotonin reuptake inhibitors and other modern antidepressants

Liver enzyme level alterations in asymptomatic persons have been observed with *fluoxetine* and *paroxetine* (581). A few reports of acute and chronic hepatitis have been attributed to the use of selective serotonin reuptake inhibitors (SSRIs) (581) (Table 33.27). In all reported cases, the liver injury subsided with drug discontinuation. *Nefazodone* has been withdrawn after being implicated in the induction of acute and subacute liver failure (582). A Spanish pharmacovigilance study examining antidepressant hepatotoxicity found that nefazodone had the highest frequency of liver injury among these drugs (28.96 per 100,000 patient-years); the comparative figures for fluoxetine, paroxetine, sertraline, and citalopram were less than 2 per 100,000 patient-years (583). Of 32 cases of liver injury analyzed by the Canadian adverse drug-monitoring program, 26 (81%) were classified as severe. About half the patients recovered after drug withdrawal but three patients progressed to acute liver failure (584), necessitating liver transplantation. Centrilobular, massive or submassive hepatic necrosis was observed on histology. Two thirds of affected persons were women aged between 30 and 70 years. Although most had been taking the drug for 3 to 6 months, early liver injury (within 4 weeks) has also been reported (585). Bioactivation of nefazodone to a reactive quinone–imine species metabolite may underlie its hepatotoxicity (586).

Trazodone has been implicated in cases of acute and chronic hepatocellular injury (587). The onset is usually within 6 months (range, 4 days to 18 months) (588). Positive rechallenge within 2 days of reinstituting the drug has been described (589). Recovery is complete within 2 months of discontinuing trazodone. Occasional

reports note the occurrence of severe hepatotoxicity with combinations of antidepressants (590) or antidepressants with other neuroleptics (43).

The liver toxicity associated with other antidepressants is summarized in Table 33.27 (591).

Table 33.27. Liver Injury Associated with Antipsychotic, Sedative-Hypnotic and Antidepressant Drugs

Drug	Nature of liver injury
ANTIPSYCHOTIC DRUGS	
Chlorpromazine	Cholestasis; vanishing bile duct syndrome
Haloperidol	Cholestasis; vanishing bile duct syndrome
Clozapine	Hepatocellular, acute liver failure
Quetiapine	Acute liver failure (single case)
Risperidone	Raised aminotransferase levels, cholestatic hepatitis
SEDATIVE-HYPNOTICS	
Barbiturates	Cholestatic or hepatocellular injury
Benzodiazepine	Hepatocellular, chronic hepatitis (bentazepam)
Chlormezanone	Cholestasis, hepatocellular, acute liver failure
ANTIDEPRESSANTS	
Monoamine oxidase inhibitors	Acute hepatitis
Tricyclic antidepressants	Hepatocellular or cholestatic; vanishing bile duct syndrome (amitriptyline, imipramine)
Tetracyclic antidepressants	Cholestasis with mianserin, maprotiline
SELECTIVE SEROTONIN REUPTAKE INHIBITORS	
Fluoxetine	Acute hepatitis, chronic hepatitis
Paroxetine	Raised aminotransferase levels, chronic hepatitis (one case)
Sertraline	Acute hepatitis when used alone and also combination with other drugs, cholestatic hepatitis
Citalopram	Cholestasis
OTHER ANTIDEPRESSANTS	
Bupropion	Raised aminotransferase levels in trials; acute hepatitis (one case),

	cholestatic hepatitis
Fluvoxamine	Acute hepatitis
Nefazodone	Raised aminotransferases, occasionally subacute liver failure
Trazodone	Acute and chronic hepatitis, cholestasis, fatal hepatic necrosis
Venlafaxine	Acute hepatitis with zone 3 necrosis, mixed liver injury; low-dose venlafaxine associated with hepatocellular injury in a patient with chronic hepatitis B

Cognition Modifiers

Tacrine (1,2,3,4-tetrahydro-9-acridinamine)

Tacrine is a reversible cholinesterase inhibitor that was formerly used in treating Alzheimer's disease. Following initial concerns about possible hepatotoxicity, a seminal observational study was conducted (592). ALT values greater than the ULN were recorded on more than one occasion in 49% of recipients; 25% had values that were more than threefold elevated, and in 2% ALT level was more than 20-fold increased (values usually >1,000 IU/L). Among patients in whom treatment was discontinued because of abnormal liver enzyme changes, biochemical resolution invariably occurred, and 88% were able to resume long-term therapy (usually at lower dose); there were no fatalities (592). Only few cases of overt hepatic disease have been reported. Histologic appearances include mild lobular hepatitis, centrilobular hepatic necrosis, steatosis, and granulomatous hepatitis (593).

The mechanism of tacrine-induced liver injury remains unclear. Hypersensitivity features were observed only infrequently and rechallenge also did not produce an exaggerated rise in ALT level. The frequency of combined glutathione-*S*-transferase genetic polymorphisms (M1 and T1) is increased in patients with tacrine-related liver injury (594), suggesting possible individual susceptibility to tacrine hepatotoxicity.

Donepezil

Donepezil hydrochloride is a specific, reversible inhibitor of centrally active acetylcholinesterase. Although postmarketing surveillance has not yet revealed significant hepatotoxicity when used alone, fulminant hepatic failure has been reported in a patient taking both donepezil and sertraline (595).

Methylphenidate

Methylphenidate is a sympathomimetic amine prescribed for attention-deficit disorders (ADDs) in children and narcolepsy and is also self-administered by intravenous injection for "recreational" use. Hepatocellular injury is documented with therapeutic (oral) and intravenous use (596,597). The latter reactions are more severe and have been associated with multiorgan

failure involving the liver, kidney, pancreas, lung, and central nervous system (597).

Pemoline

Pemoline is a psychostimulant previously prescribed for children with ADD. Clinical trials revealed mild, reversible liver injury in approximately 2% of recipients. However, instances of serious liver toxicity have since been reported to the FDA. Of 13 cases presenting with fulminant hepatic failure, 11 died or required liver transplantation (598). Symptoms were usually reported within 4 weeks but could be delayed for up to 1 year after starting therapy. Liver histology showed focal necrosis, mild steatosis, or portal inflammation (45).

Rash, fever, or eosinophilia was infrequent (599). Moreover, the observation of a similar rise in serum AT level in twins with ADD supports a hypothesis of genetically determined individual susceptibility (51). On the other hand, the existence of distinctive cases of steroid-responsive chronic liver disease resembling AIH indicates that immunoallergic mechanisms could also be involved (600). Zimmerman suggested that persons prescribed pemoline should be monitored for serum AT changes, but it is noted that the onset of acute liver failure can be extremely rapid (599). This drug is no longer considered as first-line therapy for ADD and has been withdrawn in many countries.

Other Drugs Used in the Treatment of Neurologic Diseases

Riluzole

Riluzole is a glutamate antagonist approved for the treatment of amyotrophic lateral sclerosis. Increased ALT level was observed in 1.3% to 10% of clinical trial recipients. Two cases of acute hepatitis with microvesicular steatosis have since been reported, with onset at 4 and 8 weeks after starting treatment (601). Rarely, hepatocellular injury may be delayed for as long as 6 months (602). Liver test abnormalities settle shortly after riluzole is stopped.

Dantrolene

Dantrolene is a skeletal muscle relaxant used against spasticity. The frequency of liver injury is approximately 1%. A feature has been the severity (the case fatality is approximately 28%) (603) of liver injury, which in some cases progressed even after stopping dantrolene. Most cases have been in people older than 30 years. Up to one third of affected persons had been asymptomatic, while others developed symptoms of hepatitis and jaundice. Hepatocellular necrosis, often submassive or massive, was the usual histologic characteristic (14,603). Chronic hepatitis and cirrhosis have also been observed (604). Liver tests should be performed every 2 weeks for the first 6 weeks of dantrolene therapy. The drug should be stopped if there is no clinical benefit.

Other drugs with muscle relaxant properties, which have been rarely associated with liver injury include phenyramidol (hepatocellular injury), chlormezanone (cholestasis, hepatocellular injury, and acute liver failure), and baclofen (serum AT changes) (605,606).

Tolcapone

Tolcapone is a catechol-*o*-methyl transferase (COMT) inhibitor used in the treatment of Parkinson's disease. In preclinical trials, significant ALT level elevations (>3 × ULN) were present in 1% to 3% of recipients. Tolcapone has now been implicated in at least four cases of acute liver failure (607), all in women older than 70 years. They presented with jaundice and high ALT levels. Centrilobular necrosis was observed at autopsy in one case. Ultrastructural changes included mitochondrial swelling with disruption of cristae and reduced matrix density. Patients developing severe hepatotoxicity had not been monitored as recommended, and it is noteworthy that significant liver injury has not been reported in correctly monitored recipients (608). In the United States, stringent monitoring guidelines consist of ALT testing every 2 weeks during the first year, and every 2 months thereafter. Drug withdrawal is suggested if the ALT levels exceeded the ULN. Because most cases of liver injury occur within the first 6 months, a panel of experts have suggested that less rigorous testing may be reasonable beyond this period and also that ALT levels up to two to three times ULN could be permissible (607,608).

The mechanism of tolcapone hepatotoxicity could be related to uncoupling of mitochondrial oxidative phosphorylation (609). It is relevant that *entacapone*, another COMT inhibitor does not exhibit similar toxicity at comparable concentrations (609). As compared to tolcapone, entacapone has a greater binding affinity with glucuronidation enzymes but a lower capacity for penetrating the mitochondrial outer membrane (609). Of the three recent cases of hepatic injury attributed to entacapone, there were confounding factors in two cases such as underlying alcoholic cirrhosis and use of concurrent hepatotoxic drugs. The remaining case was of mixed hepatocellular–cholestatic injury, which could possibly be linked to this drug (610). Overall, the experience with entacapone (over 300,000 patient-years) has confirmed its generally safe track record. Manufacturers advise caution in persons with impaired liver function, but routine liver test monitoring is not mandatory (611).

Drugs of Abuse

3,4-Methylenedioxymethamphetamine ("ecstasy")

Ecstasy is a widely used recreational agent that has been associated with more than 30 cases of acute liver injury, including several deaths from acute liver failure (612,613). In some European countries, 3,4-methylenedioxymethamphetamine (MDMA) is recognized as an important cause of unexplained acute liver failure in young adults, accounting for up to 25% of such cases in Spain (614). Cases may go unrecognized in Asian countries where ecstasy use is a more recent phenomenon and viral hepatitis remains very common. Liver injury was initially described as part of a hyperthermic syndrome with rhabdomyolysis, acute renal failure, and coagulopathy; this was precipitated by vigorous muscle exercise, dehydration, and increased ambient temperatures, particularly during all-night "rage" dancing (613). However, it is now clear that acute hepatitis may be the sole manifestation of MDMA toxicity; it can occur following ingestion of even a single tablet, although many of the reported cases have involved consumption of MDMA for longer periods.

MDMA is demethylated in the liver by the CYP 2D6 pathway. One initial suggestion was that persons with low-level expression of CYP 2D6 (the debrisoquine slow-metabolizer phenotype) may be susceptible to MDMA-induced hepatitis, but this has been challenged (615). Liver biopsy specimens may show acute lobular hepatitis, or zone 3 or massive hepatic necrosis, but others have observed chronic cases with hepatic fibrosis (616).

Methylene dianiline (MDA) may be confused with MDMA (617) if used in similar settings. This is illustrated by a report of cholestasis in participants of a "technoparty" who had their alcoholic beverage spiked with MDA. Unlike MDMA, which primarily causes hepatocellular injury, MDA toxicity manifests as cholestasis, as exemplified by the 1965 outbreak of jaundice in Epping, England, where bread flour had been contaminated

with MDA (618). Acute right upper quadrant abdominal pain (similar to erythromycin hepatitis) is a major feature.

Phencyclidine

Severe phencyclidine (angel dust) overdose can lead to submassive hepatic necrosis (619) accompanied by hyperthermia, rhabdomyolysis, respiratory, and renal failure (14).

Cocaine

Cocaine is a dose-dependent hepatotoxin in mice (45). Massive doses self-administered to humans cause liver injury in association with shock and other toxic phenomena (620,621). Rarely, cocaine can cause acute hepatitis without altered systemic hemodynamics and after intranasal (as opposed to parenteral) use (622). In rodents, the histologic lesions include extensive centrilobular, midzonal, or panlobular necrosis, together with microvesicular steatosis (14); gender and genetic differences, as well as the activity of CYP enzymes determine this varied histologic spectrum.

Clinical presentation of cocaine hepatotoxicity is with raised serum AT level (>10-fold in 40% of cases), nearly always accompanied by hypotension, hyperpyrexia, renal failure, myoglobulinuria, and disseminated intravascular coagulation (623). The high mortality is illustrated by one series of 39 patients among whom more than 40% died (623). Thrombotic microangiopathy is a rare complication; early recognition and institution of plasma exchange can be lifesaving for this hematologic disorder (624). The mechanism of liver injury may vary between species; in rodents it involves drug metabolism to toxic, oxy- or nitrometabolites and factors pertaining to host defenses (2). In humans, systemic hypotension and hypoxia (and possibly hyperthermia) are more likely to contribute to liver injury (623) than the direct effects of cocaine on the liver. Induction of hepatic CYP 2E1 by alcohol can potentiate the hepatotoxic effects of cocaine experimentally (625).

Drugs Used in Aversion Therapy and Treatment of Alcohol Withdrawal

Tetrabamate (Atrium)

Tetrabamate is a drug combination (i.e., febarbamate, difebarbamate, and phenobarbital) that has been used in France and Spain to treat alcohol withdrawal, tremor, and depression. *Phenobarbital* is an extremely rare cause of liver injury (cases are similar to phenytoin hepatitis) (626), while difebarbamate and febarbamate have not been previously implicated in hepatotoxicity. However, use of the combined preparation (tetrabamate) has been implicated in causing acute hepatitis (626). Onset of symptoms was between 15 days and 2 years after commencement of drug ingestion (627). Complete recovery was observed in most patients after drug cessation, but two deaths from liver failure were recorded; both fatalities were in persons who continued taking tetrabamate after the onset of symptoms. Histology shows acute hepatocellular necrosis. Other lesions described include cholestasis, microvesicular steatosis, and granulomatous hepatitis.

Hypersensitivity features were prominent in some individuals, including the presence of ANAs and/or SMAs. Therefore, immunologic idiosyncrasy may contribute to liver injury, but genetic differences in drug

metabolism could have also been responsible (628). Phenobarbital, a potent CYP enzyme inducer, could also have potentially enhanced the production of toxic metabolites from the other constituents (as in the case of VPA and the anticonvulsant hypersensitivity syndrome [RMS]) when used in drug combinations.

Disulfiram

Disulfiram has been incriminated in at least 30 instances of acute hepatocellular injury. Recovery usually occurs within 2 weeks, but liver test results can take up to 3 months to normalize. The high AT readings distinguish these reactions from alcoholic hepatitis. Rarely, acute liver failure may develop (629,630). The frequency of fatal liver injury is estimated at 1 per 30,000 treated persons per year. Three liver transplantations have been performed for disulfiram-induced acute liver failure, the youngest patient being only 16 years old (631). Baseline and serial liver test monitoring is recommended for persons receiving disulfiram therapy. *Cyanamide* produces a characteristic ground glass appearance of hepatocytes, which resembles Lafora bodies (632). This is a form of hepatic adaptation rather than liver injury. The intense immunohistochemical staining of these cytoplasmic inclusions with a polyglucosan-reactive monoclonal antibody suggests that they are derived from altered glucose metabolism (633). However, cyanamide can rarely cause acute hepatitis, and serial liver biopsy specimens from alcohol-abstinent recipients of cyanamide showed portal–portal and portal–central fibrosis (634).

Chlormethiazole

Used for years to treat alcohol withdrawal symptoms, chlormethiazole had been previously associated with only one report of acute cholestatic hepatitis. However, a recent case series suggests that chlormethiazole is associated with greater hepatotoxic potential than previously appreciated (635). All three affected persons were older than 70 years and had been taking the drug for less than 2 months for indications other than alcohol withdrawal (e.g., insomnia, depression). The biochemical profile reflected hepatocellular or mixed liver injury. One developed fatal acute liver failure; histology showed submassive necrosis. The other two patients recovered within 2 months. The authors speculated that chlormethiazole hepatotoxicity may be underreported

in the context of alcohol withdrawal because of difficulties in separating drug toxicity from the underlying alcoholic liver disease (635).

Antihistamines: H_1 Receptor Antagonists

Cetirizine

Cetrizine is a nonsedating H_1 receptor antagonist associated with transient liver enzyme abnormalities in less than 2% of recipients. Cetirizine has been implicated in four reports of liver toxicity; three were hepatocellular reactions and in the other the patient developed cholestasis (54,636). Appearance of a rash (637), eosinophilia, and presence of anti–liver/kidney microsomal antibodies in two cases (637,638) is suggestive of drug hypersensitivity. Positive rechallenge (inadvertent) has been reported (638). Other H_1 receptor blockers that have been associated with cholestatic drug reactions include *terfenadine*,*cinnarizine*, *chlorpheniramine*, and *pizotyline* (82). Temporal relationship of loratadine to two hepatocellular reactions has been described. One of these cases developed subfulminant hepatic failure and needed liver transplantation. Liver histology showed massive hepatic necrosis (639).

Gastric Acid–Lowering Agents

H_2 receptor antagonists

Rare episodes of liver injury have been reported with most H_2 receptor antagonists (640). Cross-reactivity between famotidine and cimetidine has been described (641), but there is no overall evidence of a class effect on hepatotoxicity among these structurally variable drugs. Although these agents have a good safety profile, serious hepatotoxicity has led to the withdrawal of *oxmetidine*, *ebrotidine*, and *niperotidine*.

Compared to nonusers, the relative risk for liver injury was 5.5 for cimetidine and 1.7 for ranitidine (and 2.1 for omeprazole) (642). The frequency of hepatotoxicity with cimetidine and ranitidine has been estimated at 3 to 6 per 100,000 and 1 per 100,000 prescriptions, respectively (643). The risk of liver injury with cimetidine is highest with doses exceeding 800 mg/day and at the onset of treatment. Cimetidine, ranitidine, and famotidine usually cause acute hepatitis or cholestatic hepatitis (643). Of 170 cases of H_2 receptor antagonist toxicity reported to the Australian Adverse Drug Reaction Advisory Committee, hepatotoxicity constituted 4% to 8% of reactions. Most of the affected individuals were older than 50 years (643). Hepatic reactions were more frequently reported with ranitidine than with cimetidine or famotidine. Supportive evidence of hypersensitivity features was recorded in a few patients. Others have reported Stevens-Johnson syndrome in association with ranitidine in two patients with preexisting severe liver disease; it was suggested that altered hepatic metabolism may have contributed

to the syndrome, but no data were presented to support this (644). However, others point out that markers of hypersensitivity and positive rechallenge have not been consistently demonstrated, leading them to propose an alternative hypothesis for liver injury (645). In a rat model, they showed that significant hepatocellular injury could be elicited in the presence of lipopolysaccharide-induced liver inflammation. On this basis, they suggest that similar mechanisms may underlie human ranitidine hepatotoxicity because these cases are preceded by a cluster of symptoms (e.g., fever, diarrhea, abdominal pain) that could be explained by endotoxemia. In turn, the ensuing endotoxemia-related hepatic inflammation could render the hepatocytes susceptible to ranitidine-related liver injury. Interestingly, in the same rat model, coadministration of lipopolysaccharide with famotidine, a less frequent cause of hepatotoxicity, did not elicit significant liver injury.

Proton pump inhibitors

A few reports of acute hepatitis or mixed hepatocellular–cholestatic liver injury (646) have been ascribed to the proton pump inhibitors (PPIs). Although *omeprazole* was implicated in one case of fulminant hepatic failure, concurrent acetaminophen use was not clearly excluded (see earlier) (646). Fulminant hepatic failure has also been attributed to *rabeprazole* (647). However, the role of rabeprazole in causing fulminant hepatitis appears equally less conclusive because terbinafine had also been prescribed. The authors' assertion that terbinafine hepatotoxicity is usually mild is not consistent with other data presented to the FDA (see earlier section).

Antispasmodic Drugs

Alverine

Alverine is a smooth muscle relaxant used to treat patients with irritable bowel syndrome. Two cases of acute hepatocellular injury have been reported. In the first report, the accompanying peripheral and tissue eosinophilia along with ANAs directed against a component of the nuclear envelope (lamin A and C) suggested immune-mediated liver injury. These features were absent in the second case. Both patients recovered after alverine was discontinued (648,649).

Emerging Drugs

A clinical challenge with drug-induced liver disease is provided by instances of hepatotoxicity attributable to recently introduced drugs (Table 33.28). The frequency of liver injury with these drugs will not be known until

larger studies are conducted.

Alfuzosin

Two reports of liver injury (i.e., hepatocellular and mixed hepatocellular-cholestatic) have been recorded with alfuzosin, a α_1-adrenoceptor antagonist used in treating benign prostatic hyperplasia (650,651). One of these patients had underlying chronic liver disease (651). Because alfuzosin is extensively metabolized in the liver, it is possible that the ensuing hepatotoxicity in this case was a consequence of increased drug levels. Normalization of liver test results was complete within 6 months of stopping the drug (650,651).

Bosentan

Bosentan is an endothelin antagonist prescribed for primary pulmonary hypertension, chronic heart failure, and hypertension. Dose-dependent, reversible AT level increases (up to 3 × ULN) were observed with 10% of clinical trial recipients. In a pivotal trial of patients with primary pulmonary hypertension, AT level increases of eight times ULN were observed in 3% and 7% of those assigned to the 125 mg b.i.d. and 250 mg b.i.d. arms, respectively (652). The pharmacokinetics of bosentan is unaltered by mild hepatic

function impairment (Child-Pugh A) (653) but the drug is contraindicated if more severe liver disease is present. However, an area of study is the use of bosentan in controlling portopulmonary hypertension (654,655).

Table 33.28. Liver Injury Associated with Emerging Drugs

Drug	Nature of liver injury
Alfuzosin	Hepatocellular or mixed hepatocellular–cholestatic injury
β-Interferon	Raised AT level common; clinically significant liver injury rare; can trigger autoimmune hepatitis
Bosentan	Raised AT level in at least 10% but clinically significant liver injury not described; raised AT level also observed with sitaxsentan
Orlistat	Cholestatic hepatitis, subacute liver failure (single cases only)
Imatinib mesylate	Raised AT level in 35%; acute hepatitis; massive or submassive hepatic necrosis (rare)
Infliximab	Bland cholestasis, cholestatic hepatitis with bile duct injury; acute liver failure (rare); reactivation of chronic hepatitis B
Leukotriene antagonists	
Zafirlukast	Submassive or massive hepatic necrosis
Montelukast	Acute hepatitis, cholestatic hepatitis
Ximelagatran	Raised AT in 6%; acute liver failure (relationship with drug not definitely established)

AT, aminotransferase.

In a rat model, bosentan inhibited canalicular Bsep (656). Further, coadministration of cholestasis-inducing agents such as glibenclamide (glyburide) is associated with an increased frequency of AT level elevations (29%). Bosentan-treated patients with chronic heart failure also appear to be at an increased risk of liver injury, with up to 18% showing AT level elevations in one study.

Dose-dependent AT increases were also reported with *sitaxsentan*, a selective endothelin (ET_A) receptor antagonist. The cumulative risk of developing raised AT level (up to 3 × ULN) with the 100 and 300 mg daily dose after 9 months was estimated at 8% and 32%, respectively (657).

Orlistat

This gastrointestinal lipase inhibitor was not associated with hepatic side effects in clinical trials. Abnormal AT levels were noted on routine evaluation in an obese young woman 3 weeks after beginning orlistat. She developed subacute liver failure 2 weeks later and required liver transplantation (658). Explant histology showed massive hepatic necrosis. Other reports of liver injury include an instance of fatal massive hepatic necrosis and a case of acute cholestatic hepatitis (659). Other than these three reports, orlistat has not been implicated in causing significant liver injury. Further, it has been studied as a potential therapeutic agent in patients with NAFLD.

Interferons

Interferon-α has been associated with acute "flares" in patients with hepatitis C, sometimes resulting in viral clearance (see Chapter 26). It may also rarely activate latent AIH (660). This may occur if the autoimmune etiology of chronic hepatitis has been not correctly appreciated at diagnosis, or it may represent a direct immune-mediated complication of interferon.

β-*Interferon* is increasingly used as a first-line agent in relapsing–remitting multiple sclerosis, as well as for hepatitis C in Asian countries. Analysis of pooled data from six randomized controlled trials involving over 2,800 subjects has shown that AT level increases are frequent (67% of subjects by 24 months) (661); most (75%) of these increases occur within 6 months. AT level changes settled spontaneously or after dose reduction (in 5%), and only a minority (0.4%) had their medication withdrawn. A retrospective chart review of 844 patients in British Columbia compared three different preparations of interferon-β-1b with respect to their hepatotoxic potential (662). AT level increases of up to 2.5 × ULN, 5 × ULN, and greater than 5 × ULN were found in 23% to 39%, 1.9% to 7.8%, and 0% to 1.9% of patients, respectively; the lowest figures were recorded with the intramuscular preparation of interferon-β-1b. Despite the high frequency of AT level changes observed with the β-interferons, clinically significant toxicity is rare. Of the two reported patients developing fulminant hepatic failure (622), one was also receiving nefazodone. Unmasking of AIH has also been observed with β-interferon (663,664). Baseline and periodic liver test monitoring is recommended by the manufacturers (665).

Pegylated interferon-α has a higher rate of AT abnormalities than conventional recombinant interferon-α but clinically significant hepatotoxicity has not been reported.

Imatinib mesylate

Imatinib mesylate is a tyrosine receptor kinase inhibitor that has been approved for treating chronic myeloid leukemia and advanced gastrointestinal stromal tumors. Nonhematologic adverse effects include sodium retention (resulting in pleuropericardial effusions, pulmonary edema, and ascites) and abnormal liver test results. Raised AT and/or serum bilirubin levels were observed in up to 3.5% of individuals treated. Median time to onset of raised AT and/or bilirubin levels was 100 days (666). These abnormalities usually settled with dose reduction; the drug needed to be discontinued on account of hepatotoxicity in only 0.5% of recipients. Few reports of acute hepatitis have accrued (666,667), including three cases of acute liver failure (668,669); concurrent acetaminophen intake was recorded in one of these cases. Positive rechallenge was observed in two patients with chronic myeloid leukemia (666). Histologic appearances showed acute hepatitis with extensive lobular and portal inflammation (670) or focal hepatic necrosis in less severe cases and submassive hepatic necrosis in those presenting with acute liver failure (669).

Leukotriene Antagonists

Leukotriene antagonists are a group of drugs used to treat asthma and include zileuton, zafirlukast, and montelukast. As a group, reversible increases in AT levels (>2 × ULN) were recorded in up to 3% of leukotriene antagonist (LTA) recipients in preclinical trials. Zileuton, the first member of this drug class, was implicated in a single case of acute hepatocellular injury. It has been superseded by zafirlukast and montelukast (671).

Zafirlukast

Collated global surveillance data published by the manufacturers on-line indicate over 100 cases of disturbed liver function in zafirlukast users. These include 46 reports of hepatitis and 14 of acute liver failure. The risk of developing acute liver failure was estimated at less than 1 per 100,000 patient-years. Assigning causality is difficult because details of concomitant drug intake or preexisting liver disease were not provided. Nevertheless, detailed descriptions of clinically significant hepatic injury have been published elsewhere (672,673). In these two case series, jaundice was observed in seven of nine cases. Two patients progressed to acute liver failure and needed liver transplantation. Symptoms were delayed until 6 months (range, 1.5 to 13 months) after starting zafirlukast. Inadvertent rechallenge reproduced liver injury in one case. Submassive or massive hepatic necrosis was observed in the hepatic explants. It is important to note that in persons developing acute liver failure preceding signs and symptoms of hepatitis were not always present. The apparent response to corticosteroids in four patients, three of whom had hypersensitivity features such as skin rash, and peripheral and tissue eosinophilia suggestive of immune-mediated liver injury. However, in another report the absence of hypersensitivity features and the long latent period (13 months) are more consistent with idiosyncratic liver injury (674). Cross-reactivity with montelukast has not been described (673). Periodic

monitoring of liver tests is suggested (671). However, the long latent period to liver injury in some cases, lack of preceding symptoms, and also the progression of liver injury even after drug withdrawal cast doubts on the validity of routine surveillance (671).

Montelukast

Three reports of acute hepatitis have been documented with montelukast. Time to onset of liver injury has been variable (1 month to 2 years) (675,676,677). Recovery was complete in all cases. Biochemical resolution can be slow and AT level (>2 × ULN) remained elevated for more than 1 year after drug withdrawal. Liver histology showed acute hepatitis or cholestatic hepatitis (676,677). A lymphocyte transformation test was reported as positive in one case, supporting a cause–effect relationship (676).

Infliximab

Infliximab is an anti–tumor necrosis factor monoclonal antibody used in refractory rheumatoid arthritis and Crohn's disease. Transient AT level elevations were recorded in combination with methotrexate but no major hepatic adverse effects were noted in clinical trials. Two reports of liver injury have since emerged in the early postmarketing phase. Bland reversible cholestasis was noted in one case and acute hepatitis with bile duct injury, interface hepatitis, and antibodies to double-stranded DNA in another (678,679). Smooth muscle and anti–liver/kidney microsomal antibodies were not present. Whether infliximab is directly hepatotoxic or whether it triggers autoimmune hepatocellular and bile duct damage is not clear. Two further reports of infliximab hepatotoxicity have since accrued. Fulminant hepatic failure developed in a 28-year-old woman with adult-onset Still's disease, 10 days after the second infliximab infusion. She was hepatitis B surface antigen positive, although there was no evidence of liver disease or viral replication (680). However, other potential factors to be considered in this case are the use of concurrent hepatotoxic drugs (cotrimoxazole) and also the risk of hepatic failure with Still's disease (681). There are now two well-documented cases of chronic hepatitis B reactivation after infliximab treatment in patients with Crohn's disease; a third patient with chronic hepatitis B who was receiving lamivudine was unaffected, suggesting that serologic screening and antiviral prophylaxis are appropriate for persons with chronic hepatitis B who commence infliximab treatment (682).

Ximelagatran

Ximelagatran is a thrombin inhibitor associated with increased AT levels (>3 × ULN) in 7.9% of long-term recipients, occurring most often within 6 months of commencement. Most (96%) of these biochemical changes declined slightly to less than twofold above ULN irrespective of whether the drug was continued or withdrawn. Symptomatic cases have been rare (683). Three cases of acute liver failure have been attributed to this drug but a definitive relationship has not been clearly established. At the present time, FDA approval has been withheld because of concerns about hepatotoxicity.

Hepatotoxicity of Herbal Medicines

The potential therapeutic benefits of herbal medicines in liver disease have yet to be fully defined, as reviewed elsewhere (683,684). However, the growing popularity of CAM in industrialized societies has brought about an increasing number of cases of herbal hepatotoxicity (685). Many of the herbal products have been used for centuries, in part because of their excellent safety record, so that recognition of hepatotoxicity has seemed surprising. Explanations include nonadherence to recommended doses and concurrent intake of other agents, including conventional medicines (such as acetaminophen). Toxicity may also result from the

use of newer more biologically active formulations. For instance, a 78-year-old woman developed acute hepatitis soon after using a readymade powder formulation of a fungal extract Linghzi (*Ganoderma lucidum*) despite having used this herbal compound previously for a year without toxicity (686). The only difference was that earlier treatment had been home prepared and presumably was not as concentrated as the marketed product that caused the adverse reaction. Individual susceptibility may also be an important determinant of herbal toxicity. Kava, a reasonably safe and widely used anxiolytic agent with rare hepatotoxicity, appears more likely to cause acute hepatitis (687) in whites with the slow debrisoquine phenotype (low expression of CYP 2D6) (also see "Kava"). The same phenotype has also been implicated in facilitating senna-associated hepatocellular injury; altered pharmacokinetics with prolongation of serum half-life and high metabolite levels were demonstrated (688). In comparison with these agents, others such as the pyrrolizidine alkaloids have substantial hepatotoxic potential.

Some herbal preparations (e.g., Jin Bu Huan) could incite an immunoallergic mechanism of liver injury. Others could aggravate preexisting liver disease, as noted with Ma huang, which can exacerbate AIH (689). Rarely, herbal medicines may trigger latent AIH (e.g., Dai-saiko-to, Black cohosh) (690,691,692). Such interactions between herbal medicines and preexisting liver disease are poorly understood. This is an important aspect because many patients with viral hepatitis use herbal remedies, and disclosure is not always forthcoming.

Recurring themes among reports of herbal hepatotoxicity are delayed diagnosis, product contamination, or botanical misidentification (685). The latter two issues can be rectified by exclusive use of agents prepared according to codes of good manufacturing practice; relevant legislation governing the sale of herbal products is in place in some countries, but the international availability of herbal medicines by mail and Internet ordering partly abrogates such improvements. Greater awareness of possible herbal hepatotoxicity is required on the part of physicians and the public so as to avoid the problem of delayed diagnosis; the implications of continued intake of hepatotoxic chemicals after the onset of liver injury is identical whether it is a

conventional medicinal agent or a herbal product. Natural products, often equated with "safety," may be easily overlooked by patient and doctor. Items not readily identified include skin creams, "natural" sedatives, herbal tea infusions, health tonics, and so-called energy vitalizers.

Some herbal products can complicate the management of patients with chronic liver disease by exacerbating a bleeding tendency (e.g., ginkgo) or by antagonizing the antimineralocorticoid action of spironolactone (e.g., glycyrrhizin, licorice) (685). Agents with immunostimulant actions, such as *Echinacea* and *St. John's wort*, can interfere with immunosuppressive therapy and provoke allograft rejection (693).

Herbal hepatoxicity encompasses a range of hepatic pathology from acute hepatitis, steatosis, and fibrosis, through to SOS and submassive or massive hepatic necrosis. Table 33.29 provides an updated account of contemporary agents implicated in significant liver injury, and some well-characterized and illustrative examples are briefly outlined in the subsequent text. More detailed accounts are available in recent reviews (685,693).

Chaparral

Chaparral (*Larrea tridentata*) is marketed as tablets, capsules, or herbal tea infusions. It is used as a dietary "energy" supplement and as a cure for numerous ailments ranging from chicken pox to cancer. Cholestatic hepatitis is the predominant mode of presentation, but chaparral has been associated with acute hepatitis, subacute hepatic necrosis, and acute liver failure (694). Hepatotoxicity is at least partly dose dependent; dosage recommendations were exceeded in some severe cases (694). At least three patients have developed end-stage liver disease requiring hepatic transplantation.

Germander

Used as a traditional remedy for many centuries, germander became popular as a slimming aid in the 1980s, particularly in France and Italy. More than 30 cases of liver injury were recorded, mostly in middle-aged women. Acute hepatitis developed 8 weeks after ingestion of germander capsules or herbal teas (695). Presence of ANAs, SMAs, and, transiently, antimitochondrial M2 antibodies have been recorded occasionally (696). Although most patients recovered, several fatalities from liver failure have been recorded. In some individuals, chronic hepatitis or cirrhosis was evident at presentation. Other case studies suggest that unfavorable outcomes are related more often to continued or repeated drug ingestion after the onset of liver injury. Early recrudescence of liver test abnormalities after rechallenge is suggestive of immunologic idiosyncrasy. The identification of a microsomal target for germander-induced autoantibodies is consistent with this proposal (697). On the other hand, the demonstration that the constitutive neoclerodane diterpenoids in germander are metabolized by CYP 3A enzymes to epoxides that can deplete GSH and incite oxidative stress–dependent apoptosis favors a reactive metabolite mechanism of liver injury (698).

Herbal products derived from closely allied medicinal plants *Teucrium polium L.* and *Teucrium capitatum L.*

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have also been associated with acute liver failure and acute hepatitis with bridging necrosis, respectively (699,700).

Table 33.29. Herbal Remedies and Dietary Supplements Implicated as Causing Toxic Liver Injury

Herbal remedy	Indications	Toxic constituent	Pattern of liver injury
Atractylis gummifera	Purgative, emetic, diuretic	Potassium atractylate and gummiferin	Acute liver failure
Black cohosh	Menopausal symptoms	Not known; contains diterpenoids	Acute liver failure; can trigger autoimmune hepatitis
Chaparral leaf	Multiple uses	*Larrea tridentate*	Zone 3 necrosis; massive hepatic necrosis chronic hepatitis; cholestasis
Chaso	Slimming aid	*N*-Nitrosofenfluramine	Submassive or massive hepatic necrosis, acute hepatitis
Chinese herbal medicines (see text)	Multiple indications; skin diseases; health	Many; *Dictamnus dasycarpus* present in six cases (in	Liver injury (no histology); acute hepatitis; SOS;

	tonic; viral hepatitis	combination)	vanishing bile duct syndrome
Comfrey; gordolobo yerba tea; maté tea; Chinese herbal tea	Health tonic	Pyrrolizidine alkaloids; compositae	SOS (veno-occlusive disease)
Camphor	Rubefacient	Cyclic terpenes	Abnormal liver tests, encephalopathy
Carp capsules (raw carp gallbladder)	Rheumatism, visual acuity	Cyprinol	Liver enzyme changes (no biopsy) with acute renal failure; hepatic necrosis (rats)
Cascara sagrada	Laxative	Many constituents, possibly anthraquinones	Cholestatic hepatitis, portal hypertension
Dai-saiko-to (TJ-9)[a]	Liver disease, especially chronic viral hepatitis	Scutellaria; glycyrrhizin	Acute and chronic hepatitis
Greater celandine	Gallstones	*Chelidonium majus*	Acute hepatitis; cholestatic hepatitis; fibrosis
"Green juice"	Dietary supplement	Contains vegetable extracts, micronutrients	Granulomatous hepatitis
Germander (tea, capsules)	Weight reduction; health tonic	Neoclerodane diterpenes (*Teucrium chamaedrys L.*)	Acute and chronic hepatitis; zone 3 necrosis; fibrosis, cirrhosis
Isabgol	Laxative	Not identified	Giant cell hepatitis (one report)
Jin Bu Huan Anodyne tablets	Sedation; analgesic	*Lycopodium serratum*	Acute and chronic hepatitis; steatosis; fibrosis
Kava	Anxiety disorders	Kava-lactone	Diffuse hepatocellular necrosis; cholestatic hepatitis; isolated γ-glutamyl transpeptidase increase
Kombucha "mushroom"	Health tonic	Yeast–bacteria aggregate (see text)	Liver injury (no histologic studies)
Linghzi	Multiple indications	*Ganoderma lucidum*	Acute cholestatic hepatitis
LipoKinetix	Slimming aid	? Ephedra, ? Usnic acid	Acute hepatitis, acute liver failure
Ma huang	Slimming aid	Ephedrine	Acute hepatitis;

			exacerbates autoimmune hepatitis
Margosa oil	Health tonic	*Azadirachta indica*	Reye's syndrome
Mediterranean remedy	Anti-inflammatory agent	*Teucrium polium*	Zone 3 necrosis; acute liver failure; fibrosis
Mixed preparations: Mistletoe, skullcap, valerian	Herbal tonics	Not identified; ? Scutellaria Skullcap has diterpenoids (see "Germander")	Liver injury (no histologic studies)
"Natural laxatives"	Cathartic	Senna, podophyllin, aloin	Liver injury (no histologic studies)
Oil of cloves	Dental pain	Eugenol	Dose-dependent hepatotoxin; zonal necrosis
Pennyroyal oil (squawmint)	Abortifacient; herbal drug	Pulegone metabolites	Confluent hepatocellular necrosis
Prostata	Prostatism	Saw palmetto	Hepatitis; fibrosis
Shark cartilage	Food supplement	Not identified	Abnormal liver tests (no histology)
Shou-wu-pian	Dizziness, premature graying of hair, liver disease	*Polygonum multiflorum* (?anthraquinones)	Acute hepatitis
Sho-saiko-to (TJ-9) [a]	Health tonic; viral hepatitis	Scutellaria; glycyrrhizin; others	Zonal/bridging necrosis; fibrosis; microvesicular steatosis
Usnic acid	Slimming aid	Usnic acid	Acute liver failure
Zulu remedy	Health tonic	*Callilepis laureola*	Hepatic necrosis

[a]TJ-9 is used in Japan and China; there are several alternative spellings, including Sho-saiko-to and Dai-saiko-to.
SOS, sinusoidal obstruction syndrome.

Jin Bu Huan

Jin Bu Huan has been implicated in several cases of acute hepatitis (701). Onset of symptoms occurred after a mean of 20 weeks (range, 7 to 52). Focal hepatic necrosis with numerous eosinophils, minor lobular hepatitis with microvesicular steatosis, and bridging fibrosis have been described. Resolution occurred within 8 weeks of discontinuation, but chronic hepatitis has been reported with long-term use (702). Levo-tetrohydropalmatine, the active ingredient, is structurally similar to the hepatotoxic pyrrolizidine alkaloids (see subsequent text). Despite being banned in the United States and Canada since 1994, new cases of Jin Bu Huan toxicity continue to accrue, stressing the need for continued vigilance.

Kava

Extracts of *Piper methysticum* have been used as a traditional ceremonial beverage (*Kava*, *Kava Kava*) in

South Pacific countries. Elsewhere, they are dispensed by alternative medical practitioners as anxiolytics or sedatives. Over 60 cases of hepatotoxicity have been reported worldwide (703,704,705). Most reported cases have occurred in users of alcohol or acetone extracts of the herb but traditional preparations of kava, which are aqua based, have also been rarely implicated in causing liver injury (705). The frequency of Kava-associated liver injury has been estimated at 0.24 to 0.26 per million daily doses. Many of the affected individuals are women (female-to-male ratio, 3:1). These included 11 patients with acute liver failure who required liver transplantation (704); the explants showed panacinar necrosis. In less severe cases, cholestatic or lobular hepatitis was noted. Symptoms of liver injury were reported to begin 3 to 16 weeks after starting ingestion (range, 2 weeks to 2 years; median 4.5 months). Reversible increase in GGTP level has also been recorded in Kava users (705), but this appears unrelated to the marked rise in AT level seen in patients developing liver injury and could instead reflect adaptation (microsomal enzyme induction).

The hepatotoxic potential of Kava has been disputed by phytomedicine practitioners, who cite its safe track record, and also by the presence of alcohol or concurrent hepatotoxic medications in some reports. However, there are well-documented cases occurring in the absence of such confounding factors. Moreover, positive rechallenge has been documented in at least two cases (703).

The active ingredients in kava are collectively termed *kava pyrones* (or *kavalactones*). The mechanism of liver injury is unclear but the following have been suggested: Inhibition of CYP enzymes, inhibition of cyclo-oxygenases COX-1 and COX-2, or depletion of hepatic GSH (706). An immunoallergic basis has been postulated because of the lack of dose dependency; it is further supported by the presence of autoantibodies, eosinophilia, positive lymphocyte transformation test, and also the successful use of corticosteroids in some cases. However, metabolic idiosyncrasy appears more plausible as the above "immune" phenomena are lacking in most cases (703). As noted above, the debrisoquine slow-metabolizer phenotype may predispose to Kava hepatitis in whites. However, studies involving affected Pacific islanders have not confirmed such a relationship (705), which is not surprising given the low prevalence (approximately 1%) of CYP 2D6 deficiency in these ethnic groups (706).

Pyrrolizidine alkaloids

Ingestion of these plant alkaloids is endemic in Africa (707) and Jamaica, usually as herbal tea mixtures, decoctions, or even as an enema (692). Such preparations have been associated with hepatic VOD (SOS), fibrosis, and cirrhosis. Pyrrolizidine alkaloid contamination has also been found in cases of SOS associated

with consumption of Chinese herbal teas and comfrey (97,693). In India and Afghanistan, epidemics of SOS have occurred after the contamination of wheat flour with pyrrolizidine alkaloids (708). In the acute form of SOS, the typical manifestations are abdominal pain, ascites, hepatomegaly, and raised AT levels. Liver failure can occur, even during the acute phase, but recovery is also possible. On the other hand, the prognosis is poor for individuals presenting with the chronic form of this disease; death occurs from liver failure.

Herbal slimming aids

Several cases of acute liver injury have been reported in association with herbal weight reduction remedies (709,710,711,712,713). In Japan, 12 women presented with jaundice, fatigue, diarrhea, and a biochemical picture of hepatocellular injury, 5 to 45 days after commencing *Chaso* or *Onshido* (709). Of these, two developed acute liver failure; one survived after a liver transplantation and the other died 6 weeks after admission. Pathologic findings were of massive or submassive hepatic necrosis or acute hepatitis. Over 400 cases of hepatotoxicity associated with herbal weight-loss aids were reported to the Japanese Health Ministry in 2002; Chaso and Onshido use were recorded in 21 and 135 cases, respectively. *N*-Nitrosofenfluramine has been identified as a potential hepatotoxin with these two products; it is a recognized hepatocarcinogen. Other *N*-nitrosofenfluramine–containing compounds associated with liver toxicity include *Sennomotokounou* and *LipoKinetix* (710) (see subsequent text). Over 100 cases, including 2 deaths from Sennomotokounou liver toxicity were reported to the Japanese Ministry of Health, Labour, and Welfare (713). Individual susceptibility may be important because the frequency of liver injury appears to be low; the CYP 2C19 poor-metabolizer phenotype was present in one of two patients with acute hepatitis. An immunoallergic basis was postulated for the other patient, who lacked this phenotype, but developed peripheral and tissue (liver) eosinophilia.

LipoKinetix, a dietary supplement, has been implicated in seven cases of acute hepatocellular injury in Los Angeles (710). Five of these were Japanese nationals. Onset of liver injury was within 3 months of ingestion (many within 1 month). Three patients developed acute liver failure but eventually recovered. Recovery was complete within 3 months. Liver histology was not available. The toxic ingredient was not identified in this multicomponent herbal product; potential candidates include ephedra alkaloids or usnic acid. The latter, marketed as a "fat burner," works by uncoupling oxidative phosphorylation. It has been implicated in causing acute liver failure in a 35-year-old woman (712). The disruption in mitochondrial bioenergetics and generation of oxidative stress could be central to usnic acid hepatotoxicity. Interestingly, usnic acid is also present in Kombucha "mushroom," a multipurpose tonic previously implicated in cases of hepatocellular injury (685). It has also been suggested that usnic acid could be the primary hepatotoxin in LipoKinetix-related liver toxicity. However, the absence of lactic acidosis argues against the involvement of usnic acid, a primary mitochondrial toxin (710).

Natural and Synthetic Retinoids

Hypervitaminosis A

Risk factors

Vitamin A is a dose-dependent hepatotoxin. Historically, cases of acute hypervitaminosis A with liver injury occurred among Arctic travelers who were forced to consume large quantities of polar bear liver. Today, liver injury more often follows self-medication with vitamin A preparations, although rare instances of hypervitaminosis A occur after consumption of large amounts of raw liver alone or in combination with β-carotene–rich vitamins (714,715).

Hepatic injury occurs both with acute ingestion of massive doses (>600,000 IU) and with prolonged ingestion of smaller doses (716). The mean daily dose of vitamin A in reported cases has been 96,000 IU, and the average duration of ingestion has been 7.2 years (range 11 days to 30 years), representing a mean cumulative dose of 229 million units (717). Cirrhosis has occurred in persons with a daily intake of 25,000 IU for 6 years or longer (717). It is therefore noted that up to 3% of vitamin supplements in the United States that are recommended for daily use contain 25,000 IU or more of vitamin A (716). In patients with renal failure, vitamin A dosages as low as 4,000 IU/day can lead to hepatotoxicity (718).

Diagnosis: Presentation, clinical features, and laboratory findings

In a Belgian series, only about a third of 41 cases were correctly identified at presentation; the average delay in diagnosis was 18 months (719). Hypervitaminosis A is associated with hepatotoxicity in approximately 50% of cases; other features of hypervitaminosis are usually present, such as fatigue, myalgia, bone pain, dry skin, alopecia, gingivitis, xanthosis, headache, neuropsychiatric disturbances, hypercalcemia, and growth retardation (663). The liver is often enlarged. Splenomegaly, ascites, and signs of portal hypertension are present in severe cases, but jaundice is not usually found (717).

The clinicopathologic spectrum of hypervitaminosis A–related liver disease includes minor alteration of liver enzymes, peliosis hepatis (720), noncirrhotic portal hypertension with perisinusoidal fibrosis, sclerosis of central veins, and cirrhosis. Decompensated chronic

liver disease can occur in the absence of cirrhosis and may be irreversible. Liver enzyme changes are nonspecific. Hypoalbuminemia, prolongation of prothrombin time, and hyperglobulinemia (with predominant IgM) are seen in advanced cases (717). Increased plasma level of retinyl esters (>10%) as a proportion of total serum vitamin A level (normal <8%) has been suggested as a marker of hypervitaminosis A (721), but this is not supported by the National Health and Nutrition Examination Survey (NHANES) III survey, which showed no correlation between retinyl esters and liver test abnormalities (722). Further, plasma vitamin A levels may also be normal in patients with hepatic fibrosis (717). Demonstration of increased hepatic vitamin A stores and the characteristic histologic appearances are a more reliable guide to diagnosis.

Liver pathology

Stellate cells store vitamin A in the liver. As expected, liver biopsy specimens of people with hypervitaminosis A show stellate cell hypertrophy and hyperplasia. This can give rise to sinusoidal compression and a typical honeycombed ("Swiss Cheese") appearance. Perisinusoidal fibrosis is often striking, whereas hepatocellular injury is usually minimal. Microvesicular steatosis and focal degeneration have also been reported (723). Vitamin A deposition is readily detected on fresh liver sections by the characteristic greenish autofluorescence after irradiation with ultraviolet light.

Course and management

Gradual improvement usually occurs after vitamin A is discontinued, but progression from noncirrhotic portal hypertension to cirrhosis may continue (717,724). Features of liver failure with established cirrhosis at diagnosis indicate a poor prognosis; most patients die or require hepatic transplantation (717,725). This is because turnover of vitamin A stores is very slow (half-life 58 to 286 days) (726) Abstinence from alcohol is advised because ethanol can potentiate vitamin A hepatotoxicity (716).

Synthetic retinoids

Etretinate is an aromatic synthetic retinoid (structurally unrelated to vitamin A) used in the management of psoriasis and other dyskeratotic skin disorders. Abnormal liver test results are found in up to 20% of recipients of etretinate but overt liver injury is less common (<1%). Acute and chronic hepatitis, mild cholestasis, and cirrhosis have all been reported (727,728,729). Features of drug allergy are usually not present, although eosinophilia was reported in one case (730). Recovery can be delayed because of the long tissue half-life of etretinate (120 days). Corticosteroids appeared to induce a clinical response in one report of etretinate-induced chronic hepatitis (727). It is recommended that patients taking etretinate be monitored with regular liver tests. If the liver enzyme levels are raised (>3 × ULN), the options are to either perform a liver biopsy to assess the significance of liver injury or discontinue etretinate (14).

Acitretin, the major metabolite of etretinate, has a shorter half-life (50 hours). A large study evaluating the effect of sequential liver biopsies on recipients of acitretin did not show significant histologic progression (731). However, acitretin has also been associated with a few reports of acute hepatitis, including one instance of acute cholestatic hepatitis with bile duct injury accompanied by marked hyperbilirubinemia (70

mg/dL) (732,733). Progressive fibrosis and cirrhosis can occur despite stopping acitretin (732,733). Periodic monitoring of LFTs is recommended.

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Chapter 34

Mechanisms of Drug-Induced Liver Injury

Paul B. Watkins

Key Concepts

- Most drug-induced liver injury appears to be caused by the formation of reactive metabolites from the parent drug.
- The initiating event in liver injury is often the accumulation of a reactive metabolite over a "threshold," which occurs when the rate at which the reactive metabolite is produced exceeds the rate at which it can be safely eliminated.
- Reactive metabolites can injure cells in many ways, including creating "oxidative stress" and "covalent binding," which may inhibit functioning of critical proteins or create neoantigens, stimulating an immune attack on the liver.
- The innate immune system is involved in events that kill drug-injured hepatocytes that would otherwise survive; whether a drug-injured hepatocyte dies or recovers is largely based on a balance of multiple factors that favor cell killing or cell survival.
- The liver can often adapt to low levels of liver toxicity through mechanisms that involve altered regulation of glutathione, enzymes, and transporters.
- The mechanisms common to both predictable and idiosyncratic toxicity are generally not known and can probably only be identified by studying patients who actually experienced idiosyncratic toxicity.

Drug-induced liver injury has been traditionally divided into two categories: Predictable and unpredictable (or "idiosyncratic"). Predictable liver toxins produce dose-dependent injury; essentially all patients will develop liver injury if they receive a sufficiently high dose. Drugs that are predictable toxins are generally identified in preclinical (animal) studies or during the early clinical trials specifically designed to look for dose-related toxicity. If liver toxicity occurs at doses likely to be near those required for significant therapeutic benefit, the drug is generally abandoned from further development. As a result, there are few predictable hepatotoxins in

therapeutic use today. One example of a drug that is a predictable hepatotoxin is acetaminophen. As with all predictable toxins, subtoxic exposures can generally be safely tolerated because "the dose makes the poison."

In contrast to predictable toxicity, idiosyncratic toxicity is not clearly dose related. With a true idiosyncratic toxin, only a very small fraction of the patients are susceptible to liver injury, even when receiving high doses (i.e., it is the *host* that makes the poison). Animal models, although having value in identifying predictable liver toxins in man, are generally of little help in identifying drugs capable of causing idiosyncratic toxicity. If the toxicity occurs in less than 1 in 1,000 patients receiving the drug, it might not be recognized during preapproval clinical trials and become evident only after regulatory approval and widespread usage. Recognition that an otherwise good drug can cause idiosyncratic hepatotoxicity often leads to regulatory

actions that limit access of patients to that drug, even when most patients are at no risk for toxicity. Idiosyncratic hepatotoxicity has been the major reason for regulatory actions concerning drugs, including failure to approve, marketing restrictions, and withdrawal from the market place (1).

Idiosyncratic hepatotoxicity, therefore, poses a great problem for physicians, patients, and the pharmaceutical industry. Until the mechanisms underlying idiosyncratic hepatotoxicity are identified, it is unlikely that the industry will be able to design this liability out of new drugs. The vast majority of research on the mechanisms underlying drug-induced liver injury has involved predictable hepatotoxins, particularly acetaminophen. Furthermore, it is generally assumed that the mechanisms underlying predictable toxicity are relevant to idiosyncratic toxicity, but that the latter occurs because of genetic or nongenetic factors that are present only in the susceptible patients.

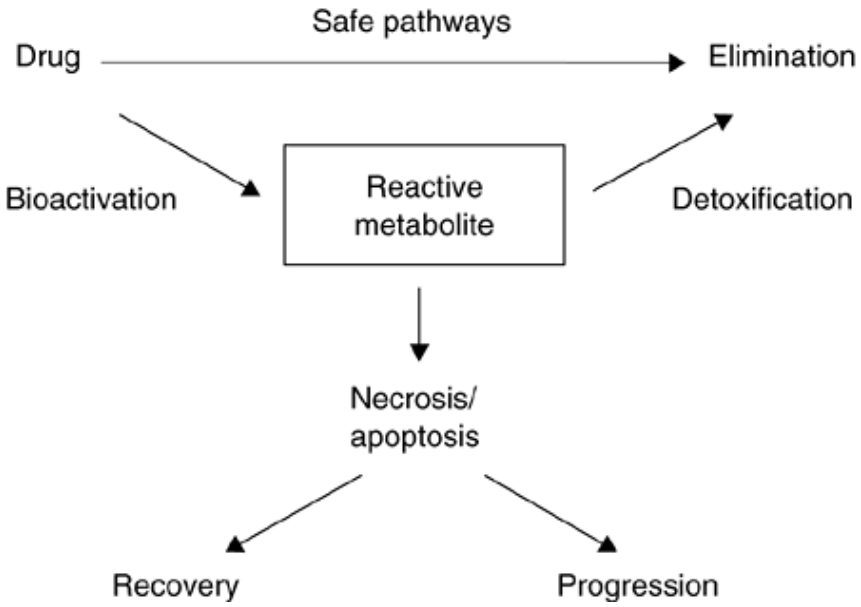
Mechanisms of Predictable Hepatotoxicity

Most predictable hepatotoxins, including environmental compounds such as aflatoxin B1, bromobenzene, and carbon tetrachloride, are relatively harmless to the liver. Predictable toxicity generally results from reactive, or toxic, metabolites generated from the parent (or "protoxin") in the liver (2,3,4,5). Formation of the reactive metabolite is generally thought of as an essential step in the cascade of events leading to hepatocyte injury. A general scheme for hepatotoxicity mechanisms involving reactive metabolites is shown in Figure 34.1. The liver is particularly susceptible to generating reactive metabolites because it is the major organ responsible for drug metabolism and elimination. Furthermore, many of the same enzymes involved in the safe elimination of drugs (nontoxic pathways) have also been implicated in creating reactive and potentially toxic metabolites.

When a reactive metabolite is involved in toxicity, it usually represents only a small fraction of the total metabolism of the drug. Hepatocytes are generally capable of efficiently detoxifying and eliminating reactive

metabolites once they are formed, thereby preventing toxicity. A generally held concept is that the initiating event is the accumulation of the reactive metabolite above some threshold. Accumulation occurs when the relative rates of production of the reactive metabolite exceeds its rate of safe removal.

The rate of production of a reactive metabolite in the hepatocyte reflects the intrinsic activity of the enzyme responsible for this conversion (termed *bioactivation* in Fig. 34.1) and the concentration of parent molecule surrounding the enzyme within the hepatocyte. The concentration of the parent molecule at the enzyme chiefly reflects (i) the competing rates of uptake into, and efflux out of, the hepatocyte and (ii) the rate of conversion of the parent molecule to safe and readily excreted metabolites (the competing *safe pathways* are shown in Fig. 34.1).



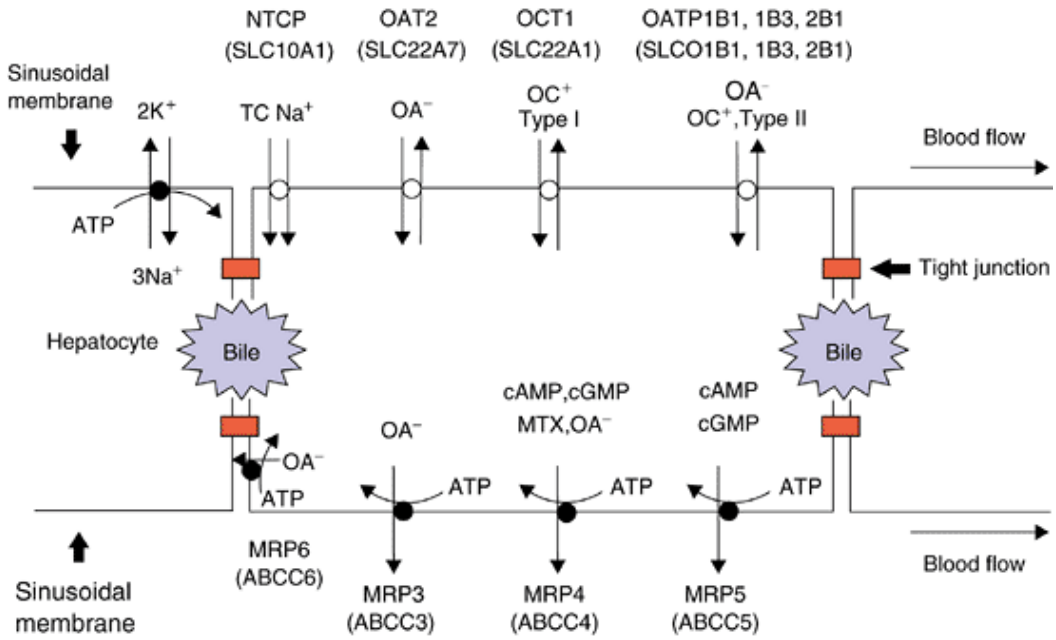
▪ **Figure 34.1** Central role of reactive metabolites in drug-induced liver injury. For most drugs, the major routes of elimination involve production of metabolites that are safely excreted and represent no threat to the liver cell because they are readily and safely excreted. Drugs that produce liver injury generally appear to do so through "bioactivation" to form reactive or toxic metabolites, which often result from relatively minor metabolic pathways. Fortunately, the liver generally has adequate ability to detoxify these metabolites, allowing their safe elimination. However, under certain circumstances, the reactive metabolite can accumulate in the hepatocyte above a threshold level for toxicity, resulting in hepatocyte damage through a variety of mechanisms discussed in the text. For a given level of hepatocyte injury, the outcome may be complete recovery or progression to death, depending on the response of the innate immune system.

Safe Pathways of Drug Metabolism and Transport

The rates of uptake and efflux of drugs (and metabolites) probably reflect largely the activities of specific transport proteins and not passive diffusion, as previously thought. There has been great progress in the identification and characterization of these transporters (Figs. 34.2 and 34.3). Uptake transporters are present on the basolateral membrane of the hepatocyte and efflux transporters are present on both the basolateral and canalicular membranes (6,7,8,9). Once inside the hepatocyte, the rate of conversion of the parent molecule to nontoxic metabolites reflects the activities of a variety of enzymes traditionally divided into two categories: Phase 1 or Phase 2. Phase 1 reactions result in a direct modification of the

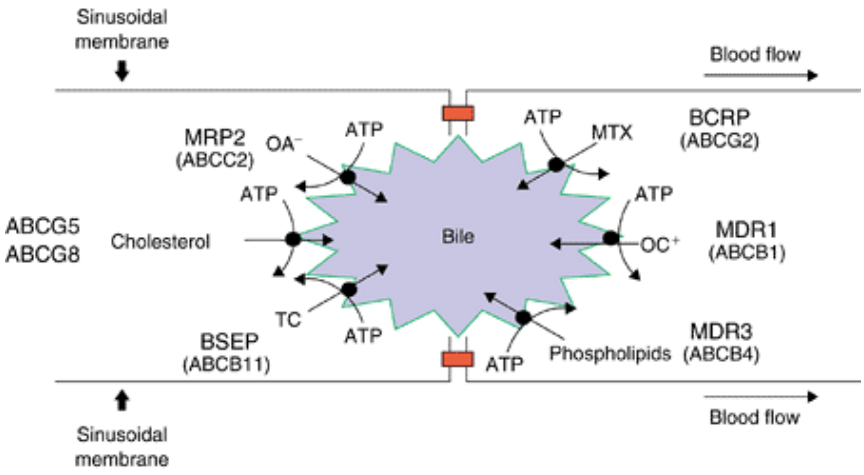
primary structure of the drug, usually resulting in insertion of an oxygen atom in the form of a hydroxyl (–OH) group. Phase 2 reactions involve covalent binding (conjugation) of the drug to polar ligands, usually glucuronic acid or sulfate, often to the hydroxyl group resulting from Phase 1

metabolism. The terms *Phase 1* and *Phase 2* refer to the fact that often, but not always, drugs must first be subjected to Phase 1 metabolism before they can be conjugated. Metabolites generated by Phase 1 and Phase 2 enzymes are generally secreted actively into bile or back into systemic circulation for elimination in the urine by the kidneys. In most instances, this appears to reflect active transport by at least many of the same proteins involved in the efflux of parent drugs (Figs. 34.2 and 34.3). Active efflux of metabolites from the hepatocyte is sometimes termed *Phase 3*.



▪ **Figure 34.2** Basolateral uptake and efflux transporters in the hepatocyte. Uptake of serum bile acids results from the action of sodium taurocholate cotransporting polypeptide (NTCP) and, to a lesser extent, organic anion transporting polypeptides (OATPs), which are also involved in uptake of many drugs. Multidrug resistance proteins (MRPs) are chiefly involved in the efflux of drugs and their metabolites, particularly glucuronide and sulfate conjugates. During the recovery phase of hepatotoxicity, due to acetaminophen or carbon tetrachloride, there is downregulation of NTCP and several OATPs, and upregulation of several MRPs, including MRP3 and MRP4. The aggregate effect of these changes, which would reduce the hepatocyte content of potentially injurious bile acids and xenobiotics, likely account in part for the adaptation to hepatotoxicity with recurrent dosing. OAT, organic anion transporter; OCT, organic cation transporter; OATP,

organic anion transporting polypeptide; TC, taurocholate; OA^-, organic anion; OC^+, organic cation; ATP, adenosine-5'-triphosphate; cAMP, cyclic adenosine-3',5'-monophosphate; cGMP, cyclic guanosine-3',5'-monophosphate; MTX, methotrexate. (Adapted from Chandra P, Brouwer KL. The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm Res* 2004;21(5):719–735.)



▪ **Figure 34.3** Canalicular efflux transporters in the hepatocyte. Bile salt excretory protein (BSEP) is the major bile acid efflux pump. Multidrug resistance-1 (MDR1) and multidrug resistance protein-2 (MRP2) are responsible for biliary excretion of most xenobiotics and their metabolites. During the recovery phase of hepatotoxicity, due to acetaminophen or carbon tetrachloride, there is upregulation of MRP2 and probably MDR1 and bile salt export pump (BSEP). These changes complement the altered regulation in basolateral transporters (Fig. 34.2) and further reduce hepatocyte content of potentially injurious bile acids and xenobiotics, thereby contributing to adaptation with recurrent dosing. OA^-, organic anion; ATP, adenosine-5'-triphosphate; MTX, methotrexate; BCRP, breast cancer resistance protein; OC^+, organic cation; TC, taurocholate. (Adapted from Chandra P, Brouwer KL. The complexities of hepatic drug transport: current knowledge and emerging concepts. *Pharm Res* 2004;21(5):719–735.)

Phase 1

It is now appreciated that most (but not all) of what was described as Phase 1 drug metabolism is the result of the activity of a large family of enzymes termed *cytochromes P-450*, now termed simply *P-450s* or *CYPs* (pronounced "sips"). Liver P-450s metabolize most drugs in use today; in many and perhaps most instances, metabolism by a P-450 is the rate-

limiting step in the elimination of the parent drug. There are relatively few P-450s important for drug metabolism (10,11), and these derive from three gene families, now termed *CYP1*, *CYP2*, and *CYP3* (12). Within each P-450 family, there are subfamilies designated by capital letters. Each subfamily generally contains multiple members, designated by Arabic numbers usually reflecting the order in which they were discovered.

Table 34.1. Characteristics Of Major Human Liver P-450S

P-450	Substrates	Inhibitors	Inducers
CYP 1A2	Tacrine Theophylline Tolcapone[a] (13) Dihydralazine[a] (14)	Fluvoxamine Cimetidine Ciprofloxacin	Cigarette smoke Charcoal-broiled foods Omeprazole
CYP 2A6	Acetaminophen[a] (15) Halothane[a] (16) Nicotine	8-Methoxypsoralen	None known
CYP 2B6	Bupropion Carbamazepine[a] (17)	None known	Rif, phen, carb, pheno, SJW
CYP 2C8	Paclitaxel Rosiglitazone	None known	Rif, phen, carb, pheno, SJW
CYP 2C9	Diclofenac[a] (18) Warfarin Tienilic acid[a] (19) Phenytoin	Fluvoxamine	Rif, phen, carb, pheno, SJW
CYP 2C19	Omeprazole Mephenytoin Diazepam Phenytoin[a] (20)	Sulfinpyrazone Ticlopidine Fluvoxamine	None identified
CYP 2D6	Debrisoquine Dextromethorphan	Fluoxetine and other selective	None identified

	Metoprolol and other β-blockers Perhexiline Amitriptyline and other neuroleptics Encainide Codeine	serotonin reuptake inhibitors Quinidine	
CYP 2E1	Acetaminophen[a] Ethanol Tolcapone[a] (13) Isoniazid[a] (21) Halothane (16)	Disulfiram Ethanol	Ethanol Isoniazid
CYP 3A4	Erythromycin Cyclosporin A Carbamazepine[a] (17) Midazolam/triazolam Lovastatin and other statins Saquinavir and other protease inhibitors Trazodone[a] (22) Nefazodone[a] (23) Troglitazone[a] (24)	Ketoconazole and other azoles Troleandomycin Ritonavir	Rif, phen, carb, pheno, SJW

[a]Drugs are metabolized to the reactive metabolites implicated in hepatotoxicity.
Rif, rifampin; phen, phenytoin, carb, carbamazepine; phenol, phenobarbital; SJW, Saint John's Wort.

A list of the major P-450s involved in human drug metabolism is shown in Table 34.1. In many instances, a single P-450 represents the major pathway of

metabolism of a drug. Many pharmaceutical companies now use high-throughput technology to identify the P-450s that metabolize compounds in development and use this information to select lead candidates for further development (25,26,27).

Phase 2

The best-studied Phase 2 enzymes involved in the elimination of nontoxic metabolites are uridine-5′-diphosphate (UDP) glucuronyltransferases and

sulfotransferases, which catalyze conjugation to glucuronic acid and sulfate, respectively. Conjugation generally results in enhanced water solubility and elimination in urine and stool. As with the P-450s, the UDP glucuronyltransferases (UGTs) and sulfotransferases arise from multigene families (28–30). The sulfotransferases are divided into five gene families, whereas the UGTs are divided into two gene families termed *UGT1* and *UGT2*. The UGT1 family contains eight enzymes (designated by Arabic numerals) and the UGT2 family contains seven enzymes. There appears to be considerable catalytic specificity of UGTs toward drugs, although such characterization is progressing relatively slowly. One problem has been that unlike P-450s, it has proved challenging to reconstitute the activity of UGTs in robust, in vitro systems.

Enzymes Involved in Generating Reactive Metabolites

It appears that the initiating step in drug-induced liver injury often involves creation of a reactive metabolite from the parent molecule in the liver (Fig. 34.1). Reactive metabolites are generally minor products of drug metabolism, and their reactivity can make them very short lived and difficult to detect in biologic systems. For this reason, identifying the precise structure of a reactive metabolite can be challenging.

P-450s are the major enzymes capable of generating reactive and potentially toxic metabolites. The identical enzymes involved in the safe metabolism of drugs are those that have been most implicated in the production of hepatotoxic metabolites (see Table 34.1 for examples). In general, P-450s are expressed in highest concentration in zone 3 hepatocytes, and this in part accounts for the predominance of pericentral (zone 3) necrosis in some forms of drug-induced liver injury (such as that due to acetaminophen) (31). Interspecies differences in P-450 catalytic activity and regulation probably contribute to the imperfect ability of preclinical animal studies to identify human hepatotoxins (32).

Several methods have been used to determine the P-450s responsible for the production of toxic metabolites. For example, if an antibody or chemical that specifically inhibits a certain P-450 causes significant reduction in the production of the reactive metabolite in liver extracts, it is assumed that this P-450 is involved (33). In some instances, it is possible to use selective P-450 inhibitors to identify the responsible P-450s in clinical studies. This strategy is suggested by the listing of specific inhibitors in Table 34.1. For example, acetaminophen is believed to cause toxicity in the liver because of the production of the metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI). The total production of NAPQI from an oral dose of acetaminophen can be estimated from the production of thiol metabolites, which are eliminated in urine. When healthy volunteers were given a dose of acetaminophen together with a CYP 2E1 selective inhibitor (disulfiram), the urinary production of NAPQI derivatives fell to an average of 69% (15), indicating a major role for CYP 2E1 in the production of this metabolite. In contrast, treatment with rifampin, which would induce multiple other P-450s (Table 34.1), did not increase urinary elimination of the thiol

metabolites (15).

Similar clinical research has been done with halothane. Severe halothane liver toxicity is thought to result from a trifluoroacetyl intermediate, which in turn leads to measurable levels of trifluoroacetyl acid (TFA) in plasma and urine (34). Plasma and urine levels of TFA fell 70% when patients receiving halothane anesthesia were pretreated with the CYP 2E1 inhibitor disulfiram (35). A small reduction in TFA production was also observed when these patients were pretreated with the CYP 2A6 inhibitor 8-methoxsalen, but no change was observed after treatment with triacetyloleandomycin (TAO), a potent inhibitor of CYP 3A4 (16). These studies indicate that the major enzyme involved in the production of the trifluoroacetyl intermediate from halothane in vivo is CYP 2E1, with a minor contribution from CYP 2A6 and no contribution from CYP 3A4.

Not all reactive metabolites are produced by P-450s. Some reactive metabolites, such as acylglucuronides (36), are products of Phase 2 metabolism. For example, glucuronide metabolites of diclofenac and valproic acid have been shown to covalently bind multiple proteins in the hepatocyte, and this covalent binding may contribute to the hepatoxicity rarely associated with these drugs (37,38).

Enzymes Involved in the Safe Elimination of Reactive Metabolites

Minor reactive metabolites are probably produced commonly during the metabolism of drugs. Fortunately, the liver generally has the ability to dispose of these

metabolites before they can cause injury. The major safe elimination pathway for reactive metabolites involves conjugation to glutathione (GSH). Although GSH is synthesized in every cell in the body, the liver is the major site of its synthesis. Under usual conditions, the hepatocyte cytosolic concentration of GSH is quite high (approximately 10 mM). GSH may conjugate to reactive metabolites spontaneously, but this reaction is usually catalyzed by the glutathione-*S*-transferases (GSTs) (39). The resultant conjugates are generally nonreactive and readily excreted into bile or urine. GSH conjugates are pumped from the liver cell into bile by transporters, particularly multidrug resistance protein-2 (MRP2) (Fig. 34.3).

There are three families of GSTs: Cytosolic, mitochondrial, and microsomal. The cytosolic GSTs are those most involved in drug metabolism and are divided into seven classes, designated α, μ, π, σ, θ, ω, and ζ (40). Each family has several members designated by Arabic numbers. For example, GSTM1 is the first of the five members of the μ gene family.

The important role of GSH conjugation has led some industry scientists to develop sophisticated methods to detect GSH conjugates in liver microsomes and whole animals (41) as an initial means of detecting reactive metabolites. However, GSH conjugates can enter bile and not be detected in whole animal studies. In addition, not all reactive metabolites depend on GSH for safe elimination. One important class of reactive

metabolites is epoxides, which can be safely eliminated through the action of microsomal epoxide hydrolase (42). There appears to be just one gene encoding this enzyme in humans.

How the Reactive Metabolites Cause Hepatotoxicity

There appear to be many potential ways by which toxic metabolites can injure the liver cells, but the most common mechanisms appear to involve covalent binding and oxidative stress. A critical target to reactive metabolites appears to be mitochondria.

Covalent Binding

A variety of experimental evidence supports the view that covalent binding of reactive metabolites to proteins can alter their function, contributing to, or causing, hepatotoxicity (5,43,44). For example, the location of covalently bound protein adducts within the acinus correlates well with the location of hepatocyte damage due to acetaminophen (45) and cocaine (46). In addition, experimental manipulations that increase or decrease the rate of covalent binding (e.g., treatment with inducers or inhibitors of specific P-450s) proportionately increase or decrease the sensitivity of the liver to toxicity (46,47,48). However, the simple magnitude of covalent binding produced by a drug does not accurately predict hepatotoxicity (37,44). For example, there exist structural analogs of acetaminophen that have relatively little hepatotoxic potential but nonetheless covalently bind to hepatic proteins at rates comparable to, or actually higher than, that observed with acetaminophen (49). In addition, under certain experimental conditions, hepatocyte injury produced by cocaine can be prevented without influencing the extent of covalent binding to hepatocyte proteins (50). It has, therefore, become clear that covalent binding to protein does not always result in toxicity. Indeed, covalent binding may in some instances represent an adaptive mechanism for the cell. For example, it has been speculated that certain cytosolic proteins identified as targets for acetaminophen covalent binding may function to protect the cell by inactivating reactive metabolites (51).

In view of the discrepancies between total covalent binding and toxicity, the idea that toxicity is caused by covalent binding to specific proteins critical to cell viability has emerged. During acetaminophen hepatotoxicity, multiple enzymes important to the hepatocyte undergo covalent modification that results in loss of catalytic activity (5). The identity of specific proteins targeted by a given metabolite probably reflects several factors. First, if the metabolite is extremely reactive, it is likely to be short lived; hence, binding can only occur to proteins that are located in close proximity to the enzyme that produced the metabolite. The closest protein to the metabolite when it is created is the enzyme that produced it; hence, when a specific P-450 is identified as the target for covalent binding, it is likely that this is the enzyme responsible for the generation of the reactive metabolite (52). If the implicated metabolite is less reactive and longer lived, it may diffuse from the site where it was formed to bind to more

distant proteins. Which specific proteins are affected also reflects the chemical nature of the metabolite and target protein. Acetaminophen and the structurally similar but not hepatotoxic compound *N*-acetyl-*m*-aminophenol (AMAP) produce comparable amounts of total covalent binding in the liver (49). However, covalent binding with acetaminophen occurs largely in mitochondria, whereas AMAP forms adducts predominantly in other cellular compartments (53). Specific binding to critical mitochondrial proteins may therefore account for the differences in hepatotoxic potential of acetaminophen and AMAP.

Oxidative Stress

The liver is the largest solid organ in the body, and its numerous metabolic functions require substantial

energy. Energy is largely provided by adenosine-5′-triphosphate (ATP), which is derived from the reduction of molecular oxygen to water. This process, termed *oxidative phosphorylation*, occurs in mitochondria. In the process of generating ATP and water, up to 5% of oxygen is converted to superoxide anion (O_2^-) and its metabolites, which are collectively termed *reactive oxygen species* (*ROS*). ROS can also be produced outside of the mitochondria as a by-product of oxidative metabolism by cytochromes P-450, particularly CYP 2E1 (54) and CYP 3A4 (55). ROS can be harmful to cells because they can react with proteins, deoxyribonucleic acid (DNA), or lipids, causing cell damage or death (56,57). For example, ROS can initiate lipid peroxidation. This is a self-propagating chain reaction in which unsaturated fatty acids of the membranes are broken down to volatile small molecules (e.g., F2-isoprostanes) that can be measured in the breath (58).

ROS usually do not accumulate in hepatocytes because there exist multiple mechanisms for their deactivation to less harmful species. Moreover, when ROS begin to accumulate, the cell mounts a coordinated "antioxidant response." This involves activation of NFR2 that mediates transcriptional activation of a cassette of genes that include those coding for glutathione synthesis and glutathione transferases, as well as certain UGTs. (59). The term *oxidative stress* has been used for the situation in which production of ROS exceeds the capabilities of antioxidant defenses, resulting in accumulation of ROS and oxidative damage to the cell (56). Oxidative stress can result in programmed cell death (apoptosis) in addition to necrosis (60).

The Role of Mitochondria as a Target for Reactive Metabolites

The mitochondria are the cell's major source of energy (ATP). With complete loss of functioning of mitochondria, the ATP-dependent Na^+/K^+ ion pumps at the plasma membrane cease to function, and the hepatocyte swells and ruptures in a process known as *necrosis*. Programmed cell death, or apoptosis, requires energy, and therefore, some mitochondrial function is required.

Loss of mitochondrial function could theoretically result from covalent binding of a reactive metabolite to key proteins involved in the maintenance of the mitochondria or from damage to mitochondrial DNA (61). Most mitochondrial proteins are encoded by genes present in the cell nucleus; however, some vital mitochondrial proteins involved in the oxidative phosphorylation are the products of mitochondrial genes. Mitochondria are relatively deficient in DNA repair enzymes, and hence DNA mutations caused by reactive metabolites (or resulting from oxidative stress) can have a greater effect on mitochondria than on other cellular organelles. For example, some antiviral drugs (e.g., fialuridine [FIAU] and azidothymidine [AZT]) are believed to have caused liver failure because of drug-induced mutations in mitochondrial genes (62,63).

Mitochondrial dysfunction can also occur as a consequence of depletion of mitochondrial GSH (64). Mitochondria are particularly susceptible to GSH depletion because they do not synthesize GSH and it must be imported from the cytosol. Mitochondria also lack catalase, and therefore rely more on glutathione peroxidase to handle ROS. The activity of glutathione peroxidase is critically dependent on the concentration of GSH within the mitochondria, with a rapid fall in activity as GSH concentrations (usually 10 to 15 mmol) fall below 2 to 3 mmol. More than 90% depletion of mitochondrial GSH therefore significantly impairs the mitochondria's ability to safely dispose of ROS. Because ROS are constantly produced in mitochondria, even in totally healthy hepatocytes, depletion of mitochondrial GSH by reactive metabolites may be sufficient to cause oxidative stress. Because the mitochondria are the preferred targets for NAPQI, which readily binds GSH, depletion of mitochondrial GSH is believed to be a mechanism for acetaminophen hepatotoxicity.

Mitochondria play a central role in apoptosis. This may reflect the notion that mitochondria evolved from a primitive bacterium that, when an oxygen atmosphere began to emerge, entered and became part of early eukaryotic cells (65). The bacterium's rudimentary machinery for oxidative phosphorylation provided the eukaryote with a means for survival in what would have otherwise been a toxic environment. The bacterium also probably benefited in most instances from the controlled environment afforded by the host cell. It has been theorized that the bacterium retained or developed the ability to kill the cell and thereby "go it alone" (66). According to this theory, as the bacteria evolved to become permanent residents in cells (i.e., mitochondria), the death mechanisms remained.

The mechanisms by which mitochondria initiate apoptosis have been largely clarified. The critical event appears to be depolarization of the inner membrane of the mitochondria, termed *mitochondrial permeability transition* (*MTP*), which results from opening of pores (67). MTP results in loss of the proton gradient required for generation of ATP. If all mitochondria are affected, necrosis will result. However, an immediate effect of MTP pore opening is the swelling of mitochondria and eventual rupture of the outer mitochondrial membrane. When mitochondria rupture, cytochrome *c* is released, and this causes activation of cytosolic caspases that initiate apoptosis. Evidence suggests that MTP may be a common

pathway for hepatocyte apoptosis resulting from diverse signals that may be involved in some forms of hepatotoxicity, including ligand binding to

plasma membrane death receptors (Fas and tumor necrosis factor receptor [TNFR]), release of calcium from the endoplasmic reticulum, and release of lysosomal enzymes (68). Apoptosis requires ATP; hence the energy status of the hepatotomy (i.e., how many mitochondria remain functional) appears to determine whether death will be by apoptosis or necrosis. Features of both necrosis and apoptosis can be present in a dying hepatocyte, termed *necrapoptosis* (69).

Progression versus Recovery

Once liver injury has begun, it can either progress to liver failure or can subside with recovery (Fig. 34.1). It was originally assumed that the critical variable here was how much damage was done by the reactive metabolite, such as the extent of critical covalent binding or extent of oxidative stress. Although this is an important variable, it has recently become clear that there are many factors that can influence the outcome that are "downstream" of the events discussed so far. These factors speed the death of injured or dying cells. Many of these factors are components of the "innate immune system" (70). The innate immune response, unlike the acquired immune response, is not antigen specific, has no memory, and can be immediately activated to attack pathogens. It is the coordinated and rapid release of certain cytokines and other biologic mediators that neutralize pathogens and, together with natural killer T cells and tissue macrophages, kill infected cells.

The innate immune response is beneficial if it limits the spread of infection. The drug-injured hepatocyte may appear as an infected cell to the innate immune system. In this case, however, the innate immune system may not be beneficial because hepatocytes that would otherwise recover are killed. This has been most clearly shown with acetaminophen in which elimination of various components of the innate immune system reduce susceptibility to acetaminophen hepatotoxicity in rodents (71). Examples of factors that increase susceptibility to acetaminophen hepatotoxicity include interferon-γ (72), macrophage inhibitory factor (MIF) (73), and TNF (74).

Factors that counteract the innate immune response are also prominent in the liver (71,75). This may reflect the fact that the liver's drug metabolizing machinery probably evolved to handle natural substances, such as natural insecticides made by edible plants or fungal products of food spoilage. Reactive metabolites are generated in the liver by many of these natural "xenobiotics," and this can result in liver injury. In this situation, as with drugs, it is probably not desirable to kill the otherwise viable hepatocytes. A reasonable hypothesis is that factors counteracting the innate immune response evolved in the liver to limit needless hepatocyte killing. Data suggest that Kupffer cells may be important in reducing response to liver toxicity (75). Cytokines that reduce susceptibility to acetaminophen hepatotoxicity include interleukin-10 (IL-10) (76) and IL-

6 (77).

Other issues that may determine whether liver injury progresses or regresses have been identified. Soluble factors released from dying hepatocytes (e.g., calpain) (78) may kill adjacent healthy hepatocytes. In addition, it has been shown that liver injury caused by one predictable toxin can be "amplified" by pretreatments that hinder the liver's ability to regenerate (79).

Although most studies of acetaminophen hepatotoxicity involve single toxic doses, recurrent dosing has been shown in rodents to greatly reduce susceptibility to subsequent otherwise toxic doses. In addition, mice treated with certain halogenated hydrocarbons develop hepatic necrosis after 1 week of treatment, but the liver injury largely resolves with continued exposure (80). This indicates that the liver can adapt in the face of true hepatotoxicity, with resolution of the injury despite continued exposure to the offending agent. This is reminiscent of transient elevations in the level of alanine aminotransferase (ALT) that reverse with continued exposure to drugs that can cause progressive liver injury (e.g., isoniazid) (1). In addition, this adaptation phenomenon may account for rare reports of narcotic/acetaminophen abusers consuming up to 65 g of acetaminophen daily without evidence of significant liver injury (81).

Adaptation to hepatotoxicity appears to involve altered regulation of the genes involved in determining reactive metabolite accumulation. For example, there is downregulation of the P-450s involved in producing NAPQI and increase in the hepatocyte content of GSH when rodents are exposed to increasing doses of acetaminophen (81). In addition, it has been shown that during the recovery phase of the acute exposure to acetaminophen or carbon tetrachloride, there is downregulation of certain basolateral uptake transporters (sodium taurocholate cotransporting polypeptide [NTCP] and several organic anion transporting polypeptides [OATPs]) and upregulation of basolateral and canalicular efflux transporters (including MRP3, MRP4, and MRP2) (82,83,84). This appears to protect the cell by preventing uptake and accelerating removal of xenobiotics and potentially toxic bile acids. These effects could result from a combination of the antioxidant response and the acute phase reaction chiefly mediated by Nrf-2 activation and IL-6, respectively (85,86). In addition, regenerating hepatocytes have reduced P-450 activity and increased GSH levels (87). A human study suggested upregulation of the several transporters, including multidrug resistance-1

(MDR1), in hepatocytes remaining after subfulminant liver injury (88).

Variation in Susceptibility to Drug-Induced Liver Injury

Increased susceptibility to hepatotoxicity could occur from any of the conditions outlined in Table 34.2. To date, most studies of susceptibility to predictable toxins have focused on the drug metabolizing enzymes and transporters implicated in the accumulation of the reactive metabolite

(89,90). For example, patients who are genetically deficient in the activities of both GSTM1 and GSTT1 appear to have increased incidence of liver injury from tacrine and valproic acid (91,92). However, significant correlation between toxicity and genetic variation, when found, appears to account for only a small fraction of susceptibility. This undoubtedly reflects the fact that accumulation of the reactive metabolite is only the initial step in the cascade of events culminating in liver injury, and each of these steps is loci for variation (Table 34.2).

Another reason for the relative lack of success in linking genetic polymorphisms to susceptibility is the prominent role played by nongenetic factors. For example, some drugs or other xenobiotics either inhibit or increase (induce) the activity of certain drug metabolizing enzymes (Table 34.1) and transporters. If safe pathways are induced or bioactivation pathways are inhibited, there should be less toxicity at a given dose of predictable toxin. Alternatively, if safe pathways are inhibited and bioactivation pathways are induced, susceptibility to toxicity should increase. This could account for the enhanced hepatoxicity of certain drug combinations, such as rifampin and pyrazinamide (93). However, because induction of metabolic pathways usually involves activation of transcription factors that result in the transcription of multiple genes (94), the effects of inducers of bioactivation pathways may be offset by simultaneous induction of safe elimination pathways (32).

An example where variation in susceptibility to toxicity can result from nongenetic factors is the enhanced susceptibility to acetaminophen toxicity observed in alcoholics. As previously discussed, the reactive metabolite mediating liver toxicity is NAPQI, which is produced predominantly by CYP 2E1 and is normally safely eliminated through conjugation with GSH.

Ethanol and Acetaminophen

Chronic consumption of ethanol appears to increase susceptibility to acetaminophen hepatotoxicity (95). Chronic ethanol consumption can increase CYP 2E1 activity (Table 34.1), and this provides an attractive explanation for incremental risks in ethanol consumers. However, early animal (96,97) and human (98) studies did not show increases in NAPQI formation when acetaminophen was given during or immediately after ingestion of ethanol. Indeed, these studies suggested that ethanol consumption actually reduces the rate of production of NAPQI, protecting the liver from toxicity. This is explained by the observation that induction of CYP 2E1 largely involves stabilization of

the enzyme (reduced degradation) and that this occurs when ethanol is bound to the enzyme as a substrate (99,100). Prolonged intoxication, therefore, results in an accumulation of CYP 2E1, but the enzyme activity is reduced because the induced CYP 2E1 is inhibited by ethanol (Fig. 34.4). When ingestion is stopped and ethanol is cleared from the liver, the accumulated CYP 2E1 is no longer inhibited and the aggregate CYP 2E1 activity is increased above baseline levels (100). However, the stabilization against degradation is also reversed, resulting in a relatively rapid fall in

enzyme activity to the preethanol exposure level. This results in a narrow window of susceptibility that probably last less than 24 hours (100). This effect of inhibition followed by transient induction is also mimicked by some other substrates of CYP 2E1, including isoniazid (101). This may account for reports of enhanced susceptibility to acetaminophen toxicity in patients treated with isoniazid (102,103).

Table 34.2. Some Potential Reasons for Increased Susceptibility to Predictable Hepatotoxicity

1. Decreased activity of safe elimination pathways
   Genetic—polymorphisms in Phase 1 and 2 enzymes, transporters
   Nongenetic—drug interactions involving enzyme/transporter inhibition, nutrition, inflammation
2. Increased activity of enzymes producing the reactive metabolite
   Genetic—gene duplication (CYP 2D6)
   Nongenetic—induction of P-450s
3. Reduced elimination of the reactive metabolite
   Genetic—polymorphisms in elimination enzymes (e.g., GSTs, EH) or transporters (e.g., MRP2)
   Nongenetic—drug interactions involving enzyme/transporter inhibition, nutrition, inflammation, cofactor depletion (GSH)
4. Variation in proteins and pathways targeted by reactive pathways
   Genetic—polymorphisms in the proteins involved in target pathways (e.g., inherited defects in oxidative phosphorylation proteins)
   Nongenetic—drug effects (e.g., aspirin inhibition of oxidative phosphorylation), nutrition, concomitant disease
5. Variation in the innate immune response
   Genetic—polymorphisms in cytokines and proteins involved in Kupffer cell and lymphocyte function
   Nongenetic—inflammation, nutrition
6. Variation in the ability to regenerate hepatocytes
   Genetic—polymorphisms in genes that are important in regeneration
   Nongenetic—effects of other drugs/xenobiotics, age, nutrition, inflammation
7. Variation in the ability to adapt to toxicity
   Genetic—polymorphisms in enzymes, transporters, and proteins involved in their regulation
   Nongenetic—effects of other drugs/xenobiotics, age, nutrition, inflammation

GST, glutathione-*S*-transferase; EH, epoxide hydrolase; MRP, multidrug resistance protein; GSH, glutathione.

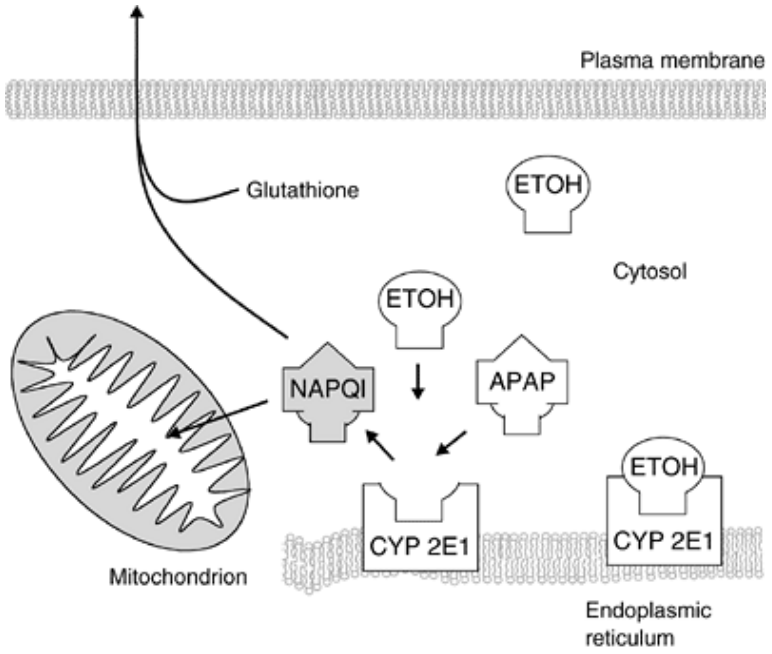
Chronic alcoholics are also likely to have reduced hepatocyte concentrations of GSH, particularly mitochondrial GSH (104), limiting the ability to safely eliminate the NAPQI once formed.

Another clinical situation that appears to increase susceptibility to acetaminophen toxicity is fasting (105). This should result in reduced GSH levels but should also cause depletion of the UGT cofactor glucuronic acid, reducing the ability to eliminate acetaminophen by nontoxic pathways. It has also been suggested that liver hypoxia due to cardiopulmonary insufficiency can increase susceptibility to acetaminophen toxicity because of reduced glucuronidation capacity (106).

Relevance of Predictable Toxicity to Idiosyncratic Toxicity

Understanding the processes involved in the production of liver toxicity from reactive metabolites has greatly improved the understanding of variation in susceptibility to predictable liver toxins, particularly acetaminophen. However, the toxicity observed still has the characteristics of a predictable toxin, such as early onset and dose dependence, within the individual. In addition, the injury to the liver remains pericentral (zone 3) necrosis, a characteristic of many direct hepatotoxins. In contrast, idiosyncratic toxicity generally begins after weeks or months of treatment and

typically involves a panacinar injury (1). These findings suggest fundamental differences between idiosyncratic and predictable hepatotoxicity. One fundamental difference may be the involvement of the acquired immune system.



▪ **Figure 34.4** Effect of ethanol on acetaminophen (APAP) toxicity. Acetaminophen is converted by CYP 2E1 to the reactive metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which is then conjugated to glutathione and safely excreted. If production of NAPQI is rapid enough to deplete available cytosolic glutathione stores, NAPQI travels to mitochondria where it covalently binds to critical proteins and depletes mitochondrial glutathione, resulting in oxidative stress. Ethanol (ETOH) binds tightly to the substrate-binding site of CYP 2E1, preventing APAP from being metabolized to NAPQI. This accounts for the observation that acutely intoxicated rodents produce less NAPQI and are resistant to APAP toxicity. However, the intracellular half-life of CYP 2E1 is prolonged when bound to ETOH, so the enzyme accumulates in an inactive form during intoxication. When ETOH consumption is ceased and ETOH removed from the liver, the accumulated CYP 2E1 becomes fully active and the rate of NAPQI formation would be increased until the CYP 2E1 levels return to baseline (probably not more than 24 hours). This effect is modest in social drinkers but can become significant when very large amounts of ethanol are consumed for prolonged periods (100).

Immunologic Mechanisms of Hepatocyte Injury

It is generally accepted that immunological mechanisms underlie the liver injury produced by some drugs (107). For example, liver injury due to halothane, phenytoin, and sulfonamides characteristically present with fever, rash, and eosinophilia, the classic clinical hallmarks of hypersensitivity (108). This type of liver injury characteristically occurs within the first month of starting therapy with the drug, similar to the time

required to become fully immunized to a vaccine. Reexposure after an episode of toxicity generally results in more rapid onset of the toxicity and greater severity of the injury, as would be expected with a hypersensitivity reaction.

There is evidence that acquired immune mechanisms may be involved in idiosyncratic hepatotoxicity even in the absence of clinical signs of hypersensitivity. For example, methyldopa-induced liver injury is not usually associated with peripheral eosinophilia, fever, or rash but recurs promptly and may be more severe on rechallenge, consistent with an immune mechanism (109). Tacrine-induced liver disease (110) also recurs promptly on rechallenge, consistent with an immunologic mechanism. However, the injury observed on rechallenge is characteristically much less severe than the original insult. The idea that the acquired immune system is involved in many forms of hepatotoxicity is also supported by lymphocyte proliferation studies. These studies are performed by isolating peripheral blood lymphocytes of patients experiencing hepatotoxicity and determining whether a subpopulation of the cells proliferate on exposure to the implicated drugs. A proliferative response implies drug stimulation of cell-mediated immunity, although it is possible that this is a consequence rather than a cause of the injury. In one study (111), 56% of 95 patients with liver injury to diverse drugs demonstrated lymphocyte proliferation in response to exposure to the implicated drug. In contrast, proliferation was not observed in any of the 35 controls who had been exposed to the same drugs without evidence of liver toxicity. In 70% of the patients with a positive test result, there were no clinical signs suggestive of hypersensitivity.

Antigens stimulating the acquired immune system

Patients with liver injury associated with several drugs characteristically have circulating antibodies to liver and kidney endoplasmic reticulum, termed *anti–liver/kidney microsomal* (*LKM*) *antibodies* (112,113). It is assumed that an acquired immune response is to a new antigen created by covalent binding between the reactive metabolite and a hepatocyte protein. Anti-LKM antibodies frequently react with P-450s (114,115,116). The current concept is that if a P-450 produces a highly reactive metabolite, the metabolite can covalently bind to, or otherwise damage, the P-450. Antibodies are formed if this altered P-450 is antigenic and gets outside the hepatocyte, from where it can be picked up by antigen-presenting cells. However, cell lysis may not be necessary for antigen recognition because P-450s appear to be present in low abundance outside the liver plasma membrane (114). It should also be noted that most proteins destined for the plasma membrane are synthesized in the endoplasmic reticulum, where most reactive metabolites are produced. Covalent modification of proteins can occur in the endoplasmic reticulum before transport to the plasma membrane (117).

There remains controversy over whether anti–P-450 antibodies actually mediate an immune attack on the liver because no one has yet convincingly shown that these antibodies can cause liver injury in a living animal model.

It remains possible that the antibodies are an epiphenomenon, appearing only after the antigens are released into circulation as hepatocytes are lysed by other mechanisms. For example, it has been noted that anesthesiologists commonly have circulating antibodies to CYP 2E1 but have no evidence of liver injury (118). However, the immune system generally will not attack healthy cells just because they contain novel antigens on their surface; it has been postulated that a second "danger signal" is required (119). The nature of this signal is not known, but it is speculated that it is present in injured cells. This is consistent with the observation that vaccines only work when given with adjuvants that cause tissue injury at the site of injection. If this theory is correct, reactive metabolites may stimulate immune attack on the liver only if they cause hepatocyte injury in addition to creating neoantigens.

Regardless of whether they mediate drug-induced liver injury, anti–P-450 antibodies can be employed in immunochemical techniques to identify the specific human P-450s that are involved in generating the reactive metabolite, even if the structure of the reactive metabolite is unknown.

It should be noted that when the lymphocyte proliferation test result is positive, the proliferation is generally most pronounced in response to the parent drug and not to its metabolites (120). This has led to the pharmacological interaction (PI) hypothesis, which states that parent drugs can initiate an immunological reaction that leads to adverse drug reactions, including hepatotoxicity (121).

Hepatotoxicity Not Related to Reactive Metabolites

Although it is generally believed that reactive metabolites are the initiating events in most forms of drug-induced liver disease, this is not always the case. For example, drugs may directly interfere with crucial hepatocyte functions, resulting in toxicity. One example of this is the interference with the homeostasis of bile acids, which can be toxic to cells, particularly to mitochondria (122). The concentration of bile acids within the hepatocyte is governed by the rates of synthesis, uptake, and efflux of bile acid, which are usually tightly regulated. If a drug interferes with this homeostasis, toxicity could result. For example, it has been demonstrated that troglitazone and bosentan inhibit the bile salt excretory protein (BSEP) (Fig. 34.2) and this may contribute to the hepatoxicity observed with these drugs (123,124,125).

The Pharmaceutical Industry and Preclinical Drug Testing

Idiosyncratic hepatotoxicity is the major single reason for regulatory actions concerning drugs. Pharmaceutical companies are currently spending many millions of dollars to improve the ways by which drug candidates are screened for hepatotoxic potential. No consensus has yet been reached and practices vary.

One approach has been to try to "humanize" preclinical testing by using cultured human hepatocytes (126) or mice that express human genes relevant to toxicity (such as human P-450s) (127). The value to these approaches is questionable because many important genes stop being expressed in cultured hepatocytes (128), it is generally not possible to maintain hepatocytes in culture for the weeks or months it characteristically takes to develop idiosyncratic toxicity, and it is statistically unlikely that the hepatocytes would have been obtained from one of the rare people actually susceptible to the toxicity. A problem with humanized mice is that we do not yet know which are the relevant genes to target.

Another approach taken is to screen compounds for the ability to form reactive metabolites. The rationale is that if formation of reactive metabolites is an essential step in idiosyncratic hepatotoxicity (Fig. 34.1), understanding the many potential processes that culminate in liver injury are irrelevant if no reactive metabolite is produced (41,129,130). The simplest way to screen compounds for the potential to produce reactive metabolites is to look for a time-dependent fall in the activity of P-450s as they metabolize the drug in in vitro systems. Such a fall may indicate the formation of a reactive metabolite that is damaging the enzyme. Another more expensive approach is to use mass spectral techniques to detect GSH adducts in liver extract incubations. A more informative (but also the most expensive) method for detecting reactive metabolite formation is to radiolabel the drug and thereby track exactly what happens to it in liver extracts or whole rats (129). Covalent binding can be easily demonstrated, and identification of the reactive metabolite is also facilitated. Once identified, chemists can attempt to modify the molecule to preserve its therapeutic effect, while reducing the ability to form the reactive metabolites.

One problem with attempting to screen reactive metabolite formation is that most drugs generate them to some degree, and some drugs will be discarded unnecessarily. This is a problem because reactive metabolite screening generally occurs relatively late in compound selection when there are only a few molecules from which to choose a "lead compound." This is because methods to screen for reactive metabolites are not high throughput, although this may soon change (131). Furthermore, some mechanisms of toxicity, such as BSEP inhibition, would not be detected by this approach.

Finally, there has been the broad application of current genomic technology to traditional preclinical toxicity testing (132). This technology allows simultaneous quantitation of thousands of messenger ribonucleic acids (mRNAs) (the "transcriptome"), proteins (the "proteome"), and endogenous metabolites (the "metabolome"). At least some companies have treated rats with known hepatotoxic and nontoxic drugs and examined time-dependent changes in the liver transcriptome, liver and serum proteome, and liver, serum, and urine metabolome. The goal is to find specific patterns that predict hepatotoxicity potential. Proteome and metabolome analyses have yet to become a standard part of preclinical testing. However, at least

several companies have selected a set of mRNA transcripts that appear to correlate with certain forms of hepatotoxicity and are routinely measuring them, along with traditional serologic markers (like ALT) and pathological evaluation, in the lead candidate selection process. This technology is also being applied to cultured hepatocytes. In one study involving cultured human hepatocytes, trovafloxacin (a fluoroquinolone antibiotic whose use was restricted because of idiosyncratic hepatotoxicity) produced transcriptome changes that were distinct from those produced by other fluoroquinolones that rarely, if ever, produce hepatotoxicity (133).

It remains to be determined whether the application of genomics technology to animal models or to cultured human hepatocytes will reduce the risk of idiosyncratic hepatotoxicity for new drugs currently in the

development pipeline. However, these techniques may speed preclinical testing by providing earlier signals of toxicity and may lead to the discovery of the new biomarkers of liver injury that can be used in the clinic.

Summary and Future Directions

The understanding of mechanisms underlying predictable liver toxicity, particularly acetaminophen-induced liver injury, has improved substantially in recent years. There has been identification of many new proteins involved in determining the extent of initial liver injury and those involved in the balance between recovery and progression. It seems logical that variation in these proteins will in part underlie susceptibility to idiosyncratic hepatotoxicity. However, testing this hypothesis will probably require studying people who have actually experienced idiosyncratic hepatotoxicity. The Drug-Induced Liver Injury Network (134) has been recently established to create a registry of such individuals and to collect serum and genomic DNA. Some susceptibility factors are likely to be highly drug specific, and meaningful study may therefore require collection of many patients with liver injury due to the same drug. However, it also seems likely that key components of susceptibility lie in the "downstream" events that may not be molecule specific and can therefore be identified by pooling subjects with toxicity to different drugs. Time will tell.

Annotated References

Bugelski PJ. Genetic aspects of immune-mediated adverse drug effects. *Nat Rev Drug Discov* 2005;4(5):410–420.

This is a good review of how drugs can stimulate the body's immune system, resulting in an adverse event. Although not specific to hepatotoxicity, most points are relevant, including a brief discussion of the "Danger hypothesis."

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2005;33(1):17–26.

This is a concise but broad overview of preclinical techniques currently used to screen compounds for hepatotoxic potential. The promise and limitations of genomic techniques are also discussed.

Evans DC, Watt AP, Nicoll-Griffith DA, et al. Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chem Res Toxicol* 2004;17(1):3–16.

This review summarizes an approach used by a major pharmaceutical company to screen compounds for reactive metabolite formation, including background on the rationale for this approach. Data obtained from the study of numerous drugs are presented.

Liebler DC, Guengerich FP. Elucidating mechanisms of drug-induced toxicity. *Nat Rev Drug Discov* 2005;4(5):410–420.

This review focuses on various ways in which drugs can cause a toxic outcome, including a concise overview of the role of signaling pathways and networks. The impact of mRNA transcript and metabonomics profiling is also presented.

Park BK, Kitteringham NR, Maggs JL, et al. The role of metabolic activation in drug-induced hepatotoxicity. *Annu Rev Pharmacol Toxicol* 2005;45:177–202.

This is a concise and current overview of the role of reactive metabolites in drug-induced liver injury, with excellent brief discussions on mechanisms underlying hepatotoxicity due to acetaminophen, halothane, isoniazid, diclofenac, and thiazolidine diones.

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Chapter 35
Wilson Disease

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Key Concepts

- Wilson disease is a genetic disorder in which copper accumulates in the liver and brain in excess of normal metabolic needs. The accumulation is based on an inherited defect in the hepatic biliary excretion of copper.
- The inheritance pattern is autosomal recessive. Homozygotes for this disorder, numbering about 1 in 30,000 of the population, inherit disease-specific mutations of both alleles of the gene for Wilson disease, *ATP7B*, on chromosome 13. The disease does not develop in heterozygotes with a mutation of a single *ATP7B* allele, and they do not require treatment.
- The diagnosis of Wilson disease is established by a combination of clinical and biochemical findings, most notably a decrease in levels of circulating ceruloplasmin, the presence of corneal Kayser-Fleischer (K-F) rings, and a hepatic copper concentration above 250 mg/g dry weight of liver.
- Molecular genetic studies demonstrating two disease-specific mutations of *ATP7B* may be used to establish a diagnosis of Wilson disease, but clinical and biochemical evaluation are needed to demonstrate phenotypic expression and to stage the disease.
- In most symptomatic patients, treatment with metal chelating agents is effective in stabilizing or reversing the disease. Asymptomatic patients may be treated with metal chelating agents or zinc salts. In all circumstances, lifelong pharmacologic treatment is required and results in excellent patient survival.
- Fulminant hepatic failure in Wilson disease or hepatic insufficiency unresponsive to medical therapy is best treated with orthotopic liver transplantation (OLT), which, by providing the liver with a normal physiologic capacity for copper excretion, is curative.

History

In 1912, while serving as a senior resident at the National Hospital for Nervous Diseases in London, Kinnier Wilson published his work *Progressive Lenticular Degeneration: a Familial Nervous Disease Associated with Cirrhosis of the Liver* as part of his dissertation for the MD degree (1). Correctly, he speculated that the brain disease, characterized by extrapyramidal features, was caused by the liver disease. However, his concept of a “morbid toxin” produced by the cirrhotic

liver, although strictly correct, could not have anticipated the much later insights into the role of the liver in copper metabolism and the vulnerability of certain areas of the brain to the toxic effects of excessive copper deposition. It was not until 33 years later that Glazebrook (2) detected a marked excess of copper in the basal ganglia of a patient dying of Wilson disease and surmised from the recognized accumulation of copper in the liver that

the inability of the liver to excrete copper was the dysfunction responsible for the lenticular degeneration, a pathogenetic association later confirmed by other workers (3,4).

In 1902 and 1903 the first descriptions of corneal pigmented rings, now recognized eponymously as Kayser-Fleischer (K-F) rings, were based on observations in patients with neurologic disease (5,6). Fleischer (7) was the first to associate three seminal features of Wilson disease—namely, corneal pigmentation, neuropsychiatric disease, and hepatic cirrhosis—and another 10 years passed before the first hypothesis that the corneal pigmentation is caused by the pathologic deposition of copper was proposed (8,9). Recognition of the value of a low serum ceruloplasmin concentration in the diagnosis of Wilson disease came from the observations of Scheinberg and Gitlin (10), who first reported this phenomenon in 96% of Wilson disease homozygotes. However, Sternlieb and Scheinberg (11) subsequently recognized that up to 20% of heterozygotes also have low ceruloplasmin concentrations without any other clinical manifestations of Wilson disease. This observation and the presence of a normal ceruloplasmin in a small minority of Wilson disease homozygotes argued against a direct pathogenetic role for ceruloplasmin in the accumulation of copper in tissues in case of Wilson disease.

With the understanding that the clinical features of Wilson disease are the result of copper toxicity in the various affected tissues of the body, the rationale for chelation therapy became apparent. The first chelation agent used for the treatment of Wilson disease, in 1951, was British anti-Lewisite (BAL), or dimercaptopropanol (12,13). This drug, which is lipophilic and therefore administered intramuscularly, enhances cupriuresis and provided the first effective therapy for a previously untreatable disorder. John Walshe of Cambridge University, while working at the Boston City Hospital, introduced the first effective oral chelation therapy in the form of penicillamine and demonstrated its cupriuretic action and role in the symptomatic improvement of patients with life-threatening features of Wilson disease (14). Sternlieb and Scheinberg (11) subsequently expanded the use of this drug to include the treatment of asymptomatic (or presymptomatic) patients with Wilson disease by showing its effectiveness in preventing disease progression. Walshe (15) proceeded to develop another, safer chelating agent, triethylene tetramine (trientine), which proved a valuable alternative agent in the treatment of those patients intolerant of the toxic effects of penicillamine. Walshe (16) was also instrumental in the initial human use of tetrathiomolybdate, currently an investigational drug in the United States.

Table 35.1. Milestones in the Genetics of Wilson Disease

Year	Milestone	References

1912	Recognition of Wilson disease as an inherited disorder	(1,21)
1953	Pattern of inheritance described as autosomal recessive	(22)
1985	Localization of disease locus to chromosome 13 by linkage with esterase D	(23)
1986–1993	Localization of the responsible gene to a specific region in chromosome 13	(24,25)
1992–1993	Identification of the gene for Menkes disease as a putative copper-transporting P-type ATPase	(26,27,28)
1993	Identification of the gene for Wilson disease, *ATP7B*, and disease-specific mutations	(29,30,31,32)
1994–present	Continued studies on disease-specific mutations and polymorphisms of *ATP7B*	(33,34)

ATPase, adenosine triphosphatase.

The possibility of preventing the toxic accumulation of copper in Wilson disease by blocking the absorption of copper with oral zinc therapy was first considered by Schouwink (17). Although oral zinc therapy would not be regarded as appropriate for the management of newly diagnosed, symptomatic Wilson disease, subsequent studies have shown it to be an effective alternative to chelation agents for long-term maintenance therapy (18,19).

A development that revolutionized the treatment of a subset of patients with Wilson disease presenting with acute liver failure was orthotopic liver transplantation (OLT), which effectively cures the disease (20). The phenotypic reversion from a diseased to a normal state in transplant recipients demonstrates the central role of the liver in Wilson disease and copper metabolism.

The recognition of Wilson disease as an inherited disorder, defined by a complex of signs and symptoms, has evolved in less than a century to the point at which we are now able to define the molecular basis for the pathophysiology of this disorder. Milestones in this process, which culminated in the identification of the gene for Wilson disease, designated *ATP7B*, and the recognition of disease-specific mutations, are reviewed in subsequent text and outlined in Table 35.1.

Genetics

Although Wilson correctly recorded the familial nature of the disease, it was Hall (21) in 1920 who demonstrated its inheritance, later shown to be autosomal recessive (22). Subsequently, the linkage of Wilson disease to the locus of the red cell *esterase-D* gene

placed the gene for Wilson disease on the long arm of chromosome 13 (23), and additional studies further delineated its chromosomal localization (24,25).

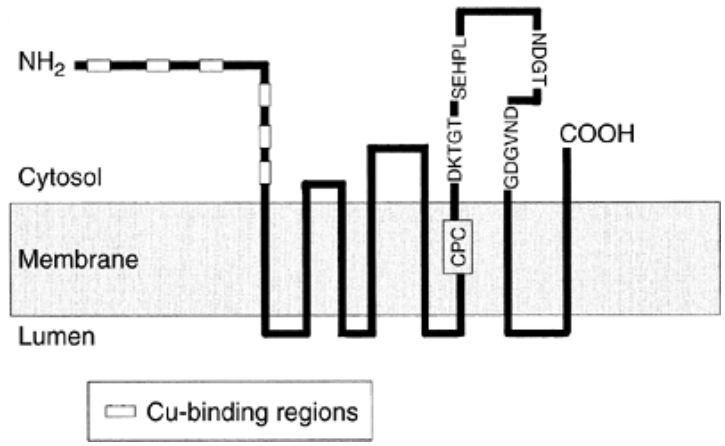
A breakthrough in the understanding of the molecular basis of the defect of copper metabolism in Wilson disease was the discovery of the gene for Menkes disease, another rare inherited disease of copper metabolism, and the identification of its gene product, ATP7A, a cation-transporting P-type adenosine triphosphatase (ATPase) involved in copper transport in many tissues (26,27,28). The extrapolation of the copper-transporting P-type ATPase to the Wilson disease model led to the hypothesis that a mutation in the gene for a liver-specific copper transporter might be responsible for the association between the defective incorporation of copper into ceruloplasmin, failure of biliary secretion of copper, and accumulation of copper in the liver.

The isolation and identification of the gene for Wilson disease, designated *ATP7B*, followed closely the discovery of the gene for Menkes disease. The identification of the specific gene was accomplished almost simultaneously by three independent laboratories (29,30,31,32). The detection of specific mutations unique to individuals with clinical and biochemically proven disease confirmed the identity of the responsible gene (29,30).

The *ATP7B* gene is contained within an approximately 80-kb region of DNA containing 22 exons (exon 22 being contained in a rare transcript); these encode an approximately 7.8-kb messenger ribonucleic acid that is highly expressed in the liver (33). Analysis of the gene sequence indicates that *ATP7B* belongs to a family of ATP-dependent metal transporters that are highly conserved through evolution (34). A schema of the *ATP7B* gene showing specific regions of known homology to ATPases and metal transporters is shown in Figure 35.1.

Screening for mutations of *ATP7B* has led to the identification of a large number (>200) of disease-specific mutations and polymorphisms of the gene (30,31,34,35,36). Most of the mutations thus far identified are point mutations that result in amino acid substitutions. However, deletions, insertions, missense, and splice site mutations have also been reported. A summary of the mutations and polymorphisms of the gene can be found in the following Web site: http://www.medicalgenetics.med.ualberta.ca/wilson/index.php. However, when particular mutations are found frequently among members of a specific population or ethnic group, direct mutational analysis is particularly useful. Haplotype analysis (polymorphism analysis of the region surrounding *ATP7B*) has proved useful for genetic screening of siblings of probands (see subsequent text). The most frequently observed point mutation, which results in a change from histidine to glutamine (H1069Q), is present in nearly 30% of patients of European descent (30,36). In only a single population in Austria has the frequency of this mutation been reported to be higher (up to 65%) (37). Most mutations are clustered about several transmembrane regions of the protein and in another region predicted to be involved in ATP binding. Some evidence indicates that mutations that result in loss of the expression of the ATP7B protein may cause more severe phenotypic expression; however, not all studies support

this conclusion. Another study suggests that expression of another gene, *APOE*, may modify the phenotype of Wilson disease. Polymorphisms in other genes involved in copper metabolism may also modify the disease phenotype, as suggested by studies of *MURR1* (38), the gene responsible for copper toxicosis in Bedlington terriers but whose function is not yet certain. Further investigations are ongoing that aim to correlate specific phenotypic presentations or manifestations of Wilson disease with *ATP7B* genotype and the expression of other potential modifying genes.

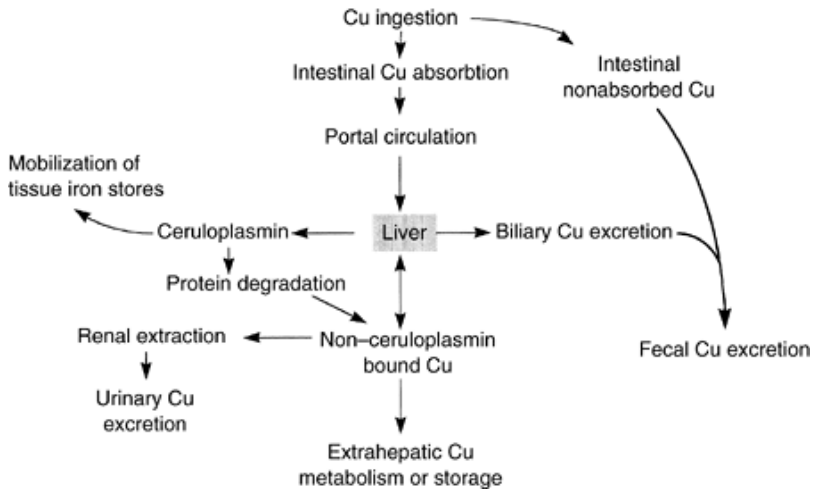


▪ **Figure 35.1** Predicted major structural features of the Wilson disease gene product (ATP7B). ATP7B, as a member of a class of heavy metal–transporting adenosine triphosphatases (ATPases), is predicted to contain the following structural features: Cysteine-rich metal-binding regions near the amino terminus, a transduction and phosphatase domain, multiple transmembrane regions with one containing the amino acids cysteine-proline-cysteine (CPC), a phosphorylation domain (DKTGT), an ATP-binding region (NDGT), a hinge region (GDGVND), and a conserved domain for metal-transforming ATPase, critical for copper transfer (SEHPL). (Adapted from Petrukhin K, Fischer SG, Pirastu M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat Genet* 1993;5:338–343, and Cox DW. Molecular advances in Wilson disease. In: Boyer JL, Ockner RK, eds. *Progress in liver disease*. Philadelphia, PA: WB Saunders, 1996:245–264, with permission.)

Pathophysiology

Copper is an essential cofactor for many enzymes and proteins and is important for the mobilization of tissue iron stores. The normal pathways for copper metabolism are outlined in Figure 35.2. Ingested copper

is extracted from the portal circulation by hepatocytes, possibly by the newly discovered cell surface human copper transporter (hCTR1) (39). Intracellular copper then interacts with low-molecular-weight ligands such as glutathione (40), metallothionein (41), and HAH1 (42), which serve as transfer or storage agents, and is subsequently used for cellular metabolic needs, incorporated into the secretory glycoprotein ceruloplasmin, or excreted into bile.



▪ **Figure 35.2** Copper metabolism and pathophysiology of Wilson disease. Dietary copper is absorbed in the proximal small intestine, whereas nonabsorbed copper or copper bound within shed enterocytes passes into the feces. Absorbed copper is bound mainly to albumin in the portal circulation, from which it is avidly extracted by hepatocytes. Hepatocellular copper is bound to ligands and used for metabolic needs, transferred to endogenous chelators, incorporated into ceruloplasmin, or excreted into bile. Biliary copper does not undergo enterohepatic recycling and is therefore excreted in the feces. In Wilson disease, biliary copper excretion is reduced, and copper accumulates within hepatocytes. The incorporation of copper into ceruloplasmin is also impaired in Wilson disease; as a result, circulating levels of this protein are decreased in most patients. When cellular stores are overloaded or after a hepatocellular injury, the amount of copper released into the circulation is increased. Extraction of the excess of non–ceruloplasmin-bound copper by the kidneys leads to an increase in urinary copper excretion and extrahepatic deposition of this metal. The dietary intake of copper is approximately 2 mg/24 hours, with intestinal absorption varying between 25% and 60% of intake. Fecal excretion is between 1 and 2 mg/24 hours, and urinary copper excretion and renal excretion do not usually exceed 50 mg/24 hours. In Wilson disease, non–ceruloplasmin-bound copper is the precursor of the excessive copper deposited in the tissues.

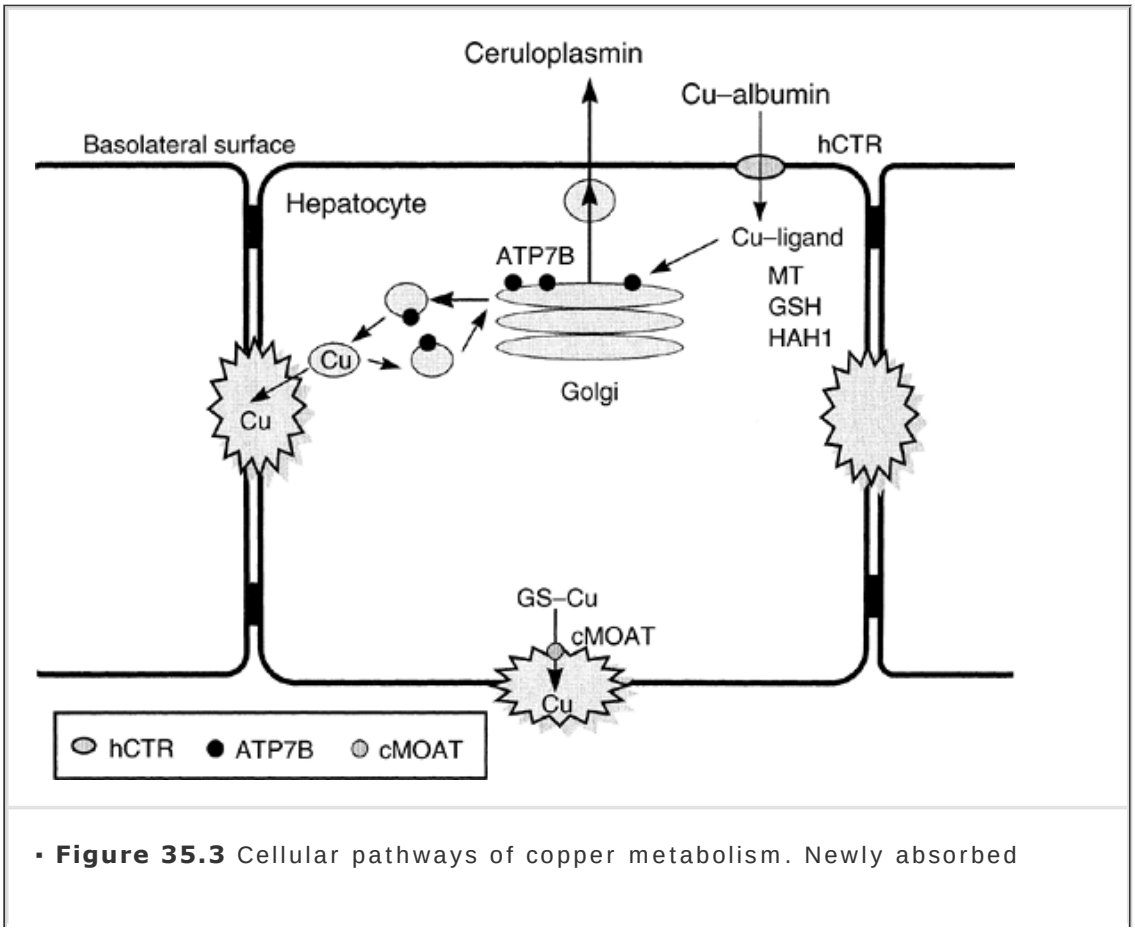
The passage of copper from hepatocytes to bile is critical for homeostasis of this metal because copper excreted into bile undergoes minimal enterohepatic recirculation (43). The transport of hepatocellular copper to bile is thought to involve a vesicular pathway that is dependent on the function of ATP7B. This protein appears to be present mainly in the *trans*-Golgi network of liver cells under basal conditions (44) (Fig. 35.3). Interestingly, in recent studies of the homologous Menkes disease protein, ATP7A, this protein was observed to alter its intracellular localization in response to increases in the level of copper (45). Although studies of ATP7B protein suggest that it also redistributes to a vesicular compartment in response to copper loading (44), how the redistribution affects the function of the protein remains to be determined. It is presumed that the vesicular pathway is critical for biliary copper excretion. However, other investigators suggest that ATP7B protein resides in a pericanalicular region and

relocates directly to the canalicular membrane in response to increased cellular copper (46). If the ATP7B protein is present at this site, it would be involved in the direct transfer of copper to bile. Whether the ATP7B protein resides in the canalicular membrane or whether copper is delivered through this site by vesicular transport, the absence or diminished function of ATP7B results in a decrease in biliary copper excretion, which is responsible for the hepatic accumulation of this metal in Wilson disease (4,47,48,49).

Ceruloplasmin is a serum glycoprotein that contains six copper atoms per molecule. It is synthesized predominantly in the liver. Copper is thought to be incorporated into apoprotein ceruloplasmin in the Golgi apparatus (50) and the copper-containing holoprotein secreted from the hepatocyte. Newly transported copper, which is used for ceruloplasmin biosynthesis, must also cross organelle membranes to enter into the protein biosynthetic pathway, a process that is dependent on ATP7B and is absent or diminished in most patients with Wilson disease. A reduction of the incorporation of copper into ceruloplasmin is believed to lead to a reduced circulating level of this protein in most patients with Wilson disease because the non–copper-containing apoprotein is less stable.

When copper accumulates beyond the cellular capacity for its safe storage, hepatocellular injury may result. Toxic effects of excess copper include the

generation of free radicals, lipid peroxidation of membranes and DNA, inhibition of protein synthesis, and alterations in the levels of cellular antioxidants (51). Recent data suggest that both hepatocellular necrosis and apoptosis may be triggered by copper-induced cell damage (52,53). When copper-induced injury occurs, the functional status of the liver is determined by the delicate balance between injury, cell death, and the regenerative capacity of liver cells.



▪ **Figure 35.3** Cellular pathways of copper metabolism. Newly absorbed

copper, loosely bound to albumin, is transported across the plasma membrane of the hepatocyte, where it is transported by a variety of ligands to the Golgi apparatus. There, the ATP7B protein serves to transport it across the Golgi into ceruloplasmin for secretion into the circulation. Excretion into bile may occur by vesicular secretion, by transcanalicular association with glutathione (GSH) through the canalicular organic anion transporter (cMOAT), or by copper-transporting adenosine triphosphatase in the canalicular membrane. hCTR, human copper transporter; MT, metallothionein;

When the capacity of the liver to store copper is exceeded, or when hepatocellular damage results in the release of cellular copper into the circulation, levels of non–ceruloplasmin-bound copper in the circulation become elevated. It is from this pool that the extrahepatic deposition of copper is thought to occur. The brain is the most critical extrahepatic site of copper accumulation, and copper-induced neuronal injury is responsible for the neurologic and psychiatric manifestations of Wilson disease and the characteristic changes on radiologic imaging studies of the brain.

Pathology

The evolution of pathologic changes in the tissues of patients with Wilson disease (see ref. 51 for review) follows the relative rates of accumulation of copper in the various body organs. Because the primary genetic defect resides in the liver and because the liver is the predominant storage organ for copper, it is not surprising that the earliest pathologic manifestations are hepatic in nature. As copper "spills" over to other organs from the liver, pathologic manifestations become evident in the brain, kidneys, eyes, and joints.

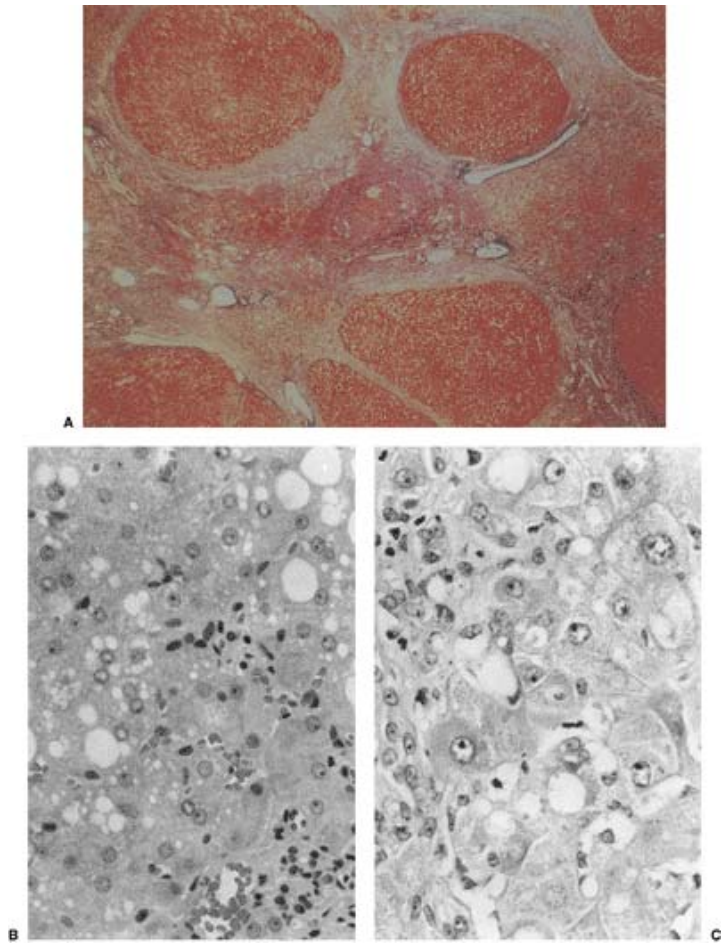
Hepatic Pathology

Macroscopically, the liver may be only mildly enlarged in the early stages of life. Later, without treatment, the liver pathology progresses in most patients to fibrosis and cirrhosis. The nodular transformation of cirrhosis is a mixed macronodular–micronodular pattern, in which the nodules may vary in color depending on the degree of copper accumulation (Fig. 35.4A). The rate of pathologic change varies greatly between patients, and in some cases steatosis and fibrosis without cirrhosis may persist for decades (54).

At a microscopic level, the evidence of copper accumulation in early infancy may be subtle and nonspecific. Diffuse cytoplasmic copper accumulation may not be visible by immunohistochemical methods for detecting copper (e.g., rhodanine, rubeanic acid). This early stage of copper accumulation is associated with macrosteatosis, microsteatosis, and glycogenated nuclei, features that may be seen in a variety of other conditions (55) (Fig. 35.4B). Sternlieb (56) emphasized the almost ubiquitous presence of distinctive mitochondrial changes at this early stage of the disease. The

ultrastructural abnormalities range from enlargement and separation of the inner and outer membranes, with widening of the intercristal spaces, to increases in the density and granularity of the matrix or replacement by large vacuoles.

Pleomorphic changes may also be seen in distorted peroxisomes and endoplasmic reticulum, with nuclei showing glycogen inclusions. With progression of the disease, copper–protein is sequestered in lysosomes, appearing as electron-dense pericanalicular structures visible on light microscopy as granules detectable by copper immunohistochemistry (Fig. 35.5).



▪ **Figure 35.4** Light microscopic findings in liver in Wilson disease. **A:** Masson trichrome stain of liver from a patient with established cirrhosis reveals broad bands of fibrosis intersecting variably sized nodules with varied staining characteristics (×80). **B:** Prominent microvesicular and macrovesicular steatosis and some inflammatory cells in a specimen from an asymptomatic patient with Wilson disease (×250). **C:** Hepatocellular ballooning and degeneration in a biopsy specimen from a patient with fulminant Wilsonian hepatitis (×250).

If the condition is untreated or unrecognized, the initial stages of Wilson disease progress to an intermediate hepatic stage; this is characterized by periportal inflammation with mononuclear cellular infiltrates, erosion of the limiting lobular plates, lobular necrosis, and bridging fibrosis, features indistinguishable from those of chronic active hepatitis of many other causes (55). Cirrhosis is virtually invariable at this stage of disease, with either a micronodular or a mixed macronodular-micronodular histologic pattern. Mallory bodies may be visible in

up to 50% of biopsy specimens.

In patients presenting with fulminant hepatic failure, parenchymal necrosis with ballooning of hepatocytes, apoptotic bodies and cholestasis, and collapse may predominate (Fig. 35.4C). In some of these individuals with fulminant failure, the liver has significant collapse and bridging fibrosis but cirrhosis may not be present (M. Schilsky, J. Lefkowitch, personal observations, 2005).

A histochemical confirmation of copper deposition may be helpful; however, a negative result does not exclude copper overload. Rhodanine and rubeanic acid may show dense granular lysosomal copper deposition in hepatocytes at the stage of cirrhotic nodular regeneration. Staining at this stage often shows marked variability from nodule to nodule (Fig. 35.6). Paradoxically, the results of immunohistochemical staining for copper are usually negative in the earlier stages of the disease, when the hepatocyte copper is diffusely distributed in the cytoplasm (57). A more sensitive stain, Timms sulfide, is more effective in detecting cytoplasmic copper-binding proteins; however, it is not routinely utilized.

Neuropathology

Macroscopically, most of the overt neuropathologic changes in advanced Wilson disease are concentrated in the lenticular nuclei. These show atrophy and discoloration, with cystic degeneration, pitting, and fissuring of the cut surfaces. Similar changes have been described in the thalamus, subthalamic region, and even the cerebral white matter (58).

Microscopically, the major pathologic changes occur in those parts of the central nervous system with the highest copper levels. Scheinberg and Sternlieb (58) calculated that the concentrations are highest in the thalamus, followed by the putamen and cerebral cortex. Neuroglial changes are the most distinctive in Wilson disease, with an increase in the number of astrocytes in the gray matter of the lenticular nuclei. Swollen glia may undergo cavitation and liquefaction, which create small cavities with an overall appearance of spongiform degeneration. Neuronal loss is accompanied by gliosis and astrocytosis and the production of glial fibrillary protein. The characteristic astrocytes seen within areas of lenticular degeneration are Alzheimer type I and II cells and, distinctively for Wilson disease, Opalski cells (59), which are large cells, up to 35 µm in diameter, with fine granular cytoplasm and slightly eccentric nuclei (single or multiple) that Scheinberg and Sternlieb (58) suggested originate from degenerating astrocytes. It is unclear at the present time whether the glial changes are secondary to the stimulation of metallothionein protein synthesis by copper in selective areas of the brain populated by protoplasmic astrocytes or whether the selective targeting of glial cells is related to other, as yet unidentified, factors.

Miscellaneous Pathologic Changes

Functional changes in the kidneys are often disproportionate to any observable changes on light microscopy. Proximal or distal tubular dysfunction leading to tubular proteinuria, bicarbonate loss, aminoaciduria, glycosuria, hyperphosphaturia, uricosuria, and hypercalciuria is common. Glomerular abnormalities, in the form of hypercellularity, basement membrane thickening, hyalinization, and fibrosis, have been described (58). Penicillamine-induced immune complex nephropathy has been described. Bone pathology and

periarticular abnormalities have been observed, accounting for osteoporosis, osteomalacia, spontaneous fractures, adult rickets, osteoarthritis, osteochondritis dissecans, chondrocalcinosis, and subchondral cyst formation (58). Involvement of the spine and knee joints is the most common distribution of skeletal and articular abnormalities.

Ophthalmologic findings include K-F rings and sunflower cataracts. The K-F rings, most marked at the upper and lower poles of the cornea, are caused by the granular deposition of elemental copper on the inner surface of the cornea in the Descemet membrane (Fig. 35.7). The rings have a golden brown or green appearance on slit-lamp examination. The sunflower cataracts, with radiating centrifugal extensions, are associated with the granular deposition of copper in the anterior and posterior lens capsule. Both the K-F rings and sunflower cataracts are reversible with effective

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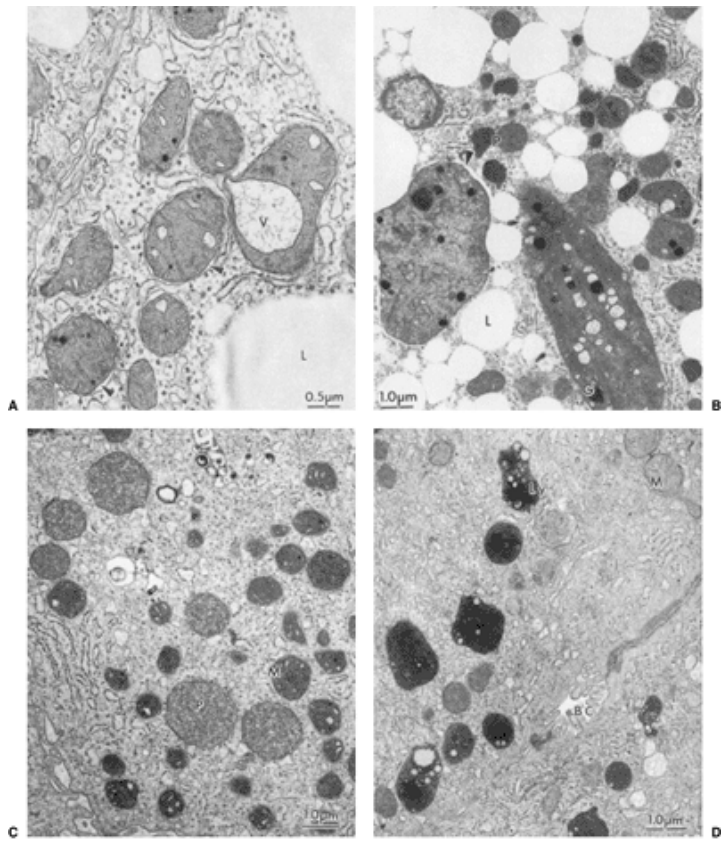
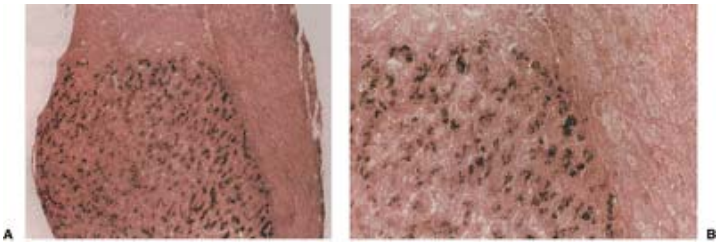
therapy for Wilson disease. Classic K-F rings are caused exclusively by the presence of excess copper in the Descemet membrane; however, rings indistinguishable from K-F rings have been seen in other forms of chronic liver disease, particularly those characterized by prolonged cholestasis (60). On slit-lamp examination, they resemble K-F rings, but chemical analysis has not confirmed that they are caused by copper deposition, although it has been hypothesized that such patients may show excessive copper retention.



▪ **Figure 35.5** Electron micrographs of fine sections of liver biopsy specimens obtained from untreated patients with Wilson disease, showing portions of hepatocytes. **A:** Ten-year-old asymptomatic girl with normal

physical findings: Aspartate aminotransferase, 76 IU/L; alanine aminotransferase, 55 IU/L; serum ceruloplasmin, 21.4 mg/dL; hepatic copper, 1,258 mg/g of dry tissue. Some of the mitochondria display vacuoles (*V*) with granular material. Note separation of inner from outer membrane (*arrowheads*), with creation of an enlarged intermembranous space. *L*, lipid droplet. **B:** Nineteen-year-old woman with a history of progressive fatigue, found to have Kayser-Fleischer rings 2 months after an episode of hemolytic anemia with jaundice: Serum ceruloplasmin, 12.7 mg/dL; hepatic copper, 591 mg/g of dry tissue. The mitochondria are markedly pleomorphic, some displaying multiple, pathognomonic abnormalities: Gigantism, increased matrical density, separation of inner from outer membrane (*arrowhead*), vacuoles, dilated cristae, crystals, and enlarged dense granules (*G*). *P*, peroxisomes. **C:** Ten-year-old asymptomatic girl with serum ceruloplasmin level below 1 mg/dL and a hepatic copper concentration of 1,029 mg/g of dry tissue. Mitochondria (*M*) display dilated cristae; the markedly enlarged peroxisomes (*P*) with grainy matrices are strikingly abnormal. **D:** In a 20-year-old woman with severe neurologic Wilson disease, the hepatocellular cytoplasm appears virtually normal except for the abundance of electron-dense, peribiliary, lysosomal granules (*Ly*). *BC*, bile canaliculus; *M*, mitochondria.

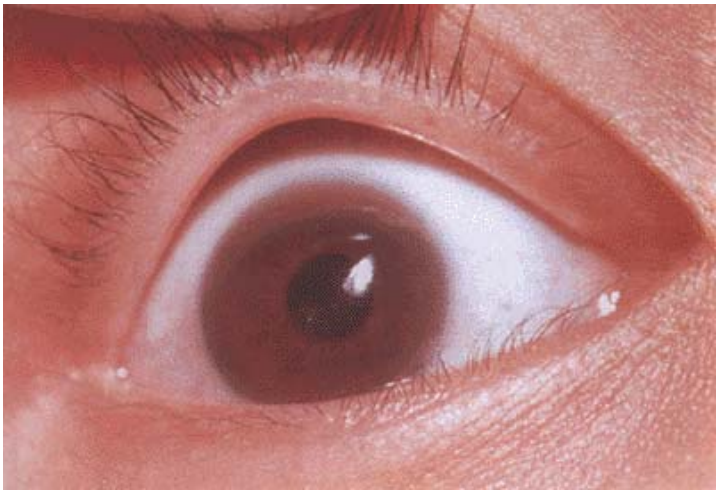


▪ **Figure 35.6 A,B:** Liver section stained with rhodanine to show nodule with heavy lysosomal copper deposition in adjoining liver tissue with minimal copper staining; **(A)** low power ×110 and **(B)** high power ×200.

Diagnosis

The diagnosis of Wilson disease should be considered in any person aged between 3 and 40 years with unexplained hepatic, neurologic, or psychiatric disease, although rare cases have been diagnosed in persons in the sixth, seventh, and even eighth decades of life (54). In particular, this diagnosis must be excluded unequivocally in children or young adults who present with unusual extrapyramidal or cerebellar motor disorders, atypical psychiatric disease,

unexplained hemolysis, or elevated liver enzyme levels or other manifestations of liver disease, with or without a family history of liver or neurologic disease. The failure to do so will lead to unnecessary and preventable demise. In most cases, the diagnosis can be made by a combination of clinical and biochemical testing. In practice, three levels of tests may be undertaken to confirm the diagnosis of Wilson disease (Table 35.2). The presence of K-F rings and a reduced serum concentration of ceruloplasmin are sufficient to establish the diagnosis. However, in the absence of K-F rings, it is necessary to proceed to a liver biopsy for a quantitative copper determination to confirm the diagnosis.



▪ **Figure 35.7** Kayser-Fleischer ring in a 17-year-old patient with neurologic symptoms of Wilson disease.

Molecular testing is currently available for Wilson disease. There are two types of testing that may be employed. Haplotype analysis that looks for familial inheritance of polymorphisms around the *ATP7B* gene should be considered for use in screening the siblings of affected persons. Direct mutational analysis is now available for de novo diagnosis of Wilson disease. Newer methodology has made the molecular screening of the entire coding region of *ATP7B* less cumbersome and time consuming, although some targeting of specific exons with higher frequencies of mutations may make sense while testing in individual populations in which specific mutations are present at higher frequency. There is a need to identify disease-specific mutations of the gene to firmly establish the diagnosis without biochemical testing. Although molecular studies may detect the disease, it may still be useful to fully characterize patients with the disorder who are detected by standard testing to better understand the degree of disease involvement, as well as to provide confirmation of the diagnosis. Given the current relative high cost and complexity of this testing, molecular testing will not readily be used as a screening test for Wilson disease. However, for patients in whom there is

difficulty in determining the diagnosis by clinical and biochemical testing, molecular testing should prove extremely useful.

Table 35.2. Diagnostic Tests in Wilson Disease

Level 1	Level 2	Level 3
Low serum ceruloplasmin (a)	Liver copper concentration (c)	Incorporation of radiocopper into ceruloplasmin
Slit-lamp examination for K-F rings	—	Ultrastructural studies of hepatocytes
Raised serum-free copper level (b)	24-h urine copper (d)	Molecular genetic studies for Wilson disease

Normal values: (a) 20–50 mg/dL; (b) <10 mg/dL; (c) <250 mg/dL; (d) <50 µg/24 hours.
Wilson disease: (a) <20 mg/dL; (b) >25 mg/dL; (c) >250 µg/g dry weight; (d) >100 µg/24 hours.
K-F, Kayser-Fleischer.

Concentration of Ceruloplasmin

A test to determine the serum concentration of ceruloplasmin is routinely available in all clinical laboratories. It is most useful, when measured by means of enzymatic methods, to determine oxidase activity because this best reflects the copper content of the protein, although immunologic assays are frequently utilized. The normal range is 20 to 50 mg/dL. About 95% of homozygotes with Wilson disease have values of less than 20 mg/dL. Up to 5% of all homozygotes and up to 15% to 50% of persons with liver disease may have normal levels, which is defined as concentrations above 20 mg/dL (58,61). In some cases, normal levels are present in patients with active liver disease, presumptively as a consequence of acute-phase responses in the liver or estrogen supplementation. Homozygotes rarely have serum concentrations exceeding 30 mg/dL. Low serum concentrations of ceruloplasmin may also be observed in hypoproteinemic states, such as protein-calorie malnutrition, nephrotic syndrome, protein-losing enteropathy, and other forms of severe decompensated liver disease, and in up to 20% of asymptomatic heterozygous carriers of the gene for Wilson disease. Rarer causes of serum ceruloplasmin deficiency include hereditary aceruloplasminemia and Menkes disease.

Concentration of Circulating Copper not Bound to Ceruloplasmin

The total concentration of copper in plasma or serum represents ceruloplasmin-bound copper plus non–ceruloplasmin-bound (“free”) copper, which is bound mainly to albumin, peptides, or amino acids. Because the former is reduced in proportion to the degree of hypoceruloplasminemia, the total copper may be low in the face of a typically raised free copper concentration. To calculate the latter, the ceruloplasmin copper content (approximately the ceruloplasmin level in

milligrams per deciliter multiplied by 3) is subtracted from the total copper content (in micrograms per deciliter). The value for total plasma copper ranges from 80 to 120 mg/dL and the value for non–ceruloplasmin-bound copper is usually 10% of the total value (8 to 12 mg/dL). In Wilson disease, levels of non–ceruloplasmin-bound copper are typically above 25 mg/dL in symptomatic patients before treatment.

Slit-Lamp Detection of Kayser-Fleischer Rings

It is essential that all patients in whom Wilson disease is suspected undergo a slit-lamp examination, performed by an experienced ophthalmologist, for the detection of K-F rings. These rings are present in patients with neurologic disease, with only rare exceptions, but they may be absent particularly in younger patients with only hepatic manifestations (Fig. 35.7). Another finding that may suggest Wilson disease is the presence of sunflower cataracts, also best observed by slit-lamp examination.

Concentration of Copper in the Liver

Normal concentrations of copper in the liver rarely exceed 50 µg/g dry weight of liver. Most patients homozygous for Wilson disease have levels above 250 µg/g, whereas the concentration of copper in the liver of heterozygotes, although commonly elevated above normal, typically does not exceed 250 µg/g (58). The hepatic copper concentrations may also be elevated in other liver diseases, particularly chronic cholestatic diseases such as primary biliary cirrhosis and primary sclerosing cholangitis. However, these disorders are usually readily distinguishable from Wilson disease on the basis of serologic and histologic criteria.

A recent study by Ferenci et al. (62) suggested that in some individuals confirmed to have Wilson disease by molecular studies, hepatic copper content does not reach 250 µg/g dry weight liver. On the basis of these results, these investigators proposed a lower threshold for considering Wilson disease—70 µg copper/g dry weight liver. However, no data are presented in this study on heterozygotes in whom hepatic copper may easily exceed this threshold. Therefore, the previous cutoff of 250 µg copper/g dry weight liver may better

differentiate heterozygotes and homozygotes with Wilson disease, but this must not be the sole criterion for excluding the diagnosis if there is appropriate histology or clinical signs of disease. In patients for whom the diagnosis remains uncertain despite extensive clinical and biochemical evaluation, molecular studies will help in confirming or refuting the diagnosis of Wilson disease.

Biopsy specimens for a quantitative copper determination should be obtained with a (Tru-Cut or Jamshidi) needle and placed dry in a copper-free vessel. About 1 cm of a 1.6-mm-diameter core of liver should be dried overnight at 56°C in a vacuum oven or, alternatively, frozen immediately before being shipped to a laboratory specializing in microchemical analysis for copper. The remainder of the specimen can be fixed in the usual manner for histopathologic examination, with the option to perform immunohistochemical staining for copper. In asymptomatic patients, the hepatic copper level is higher than that in patients with established cirrhosis. Specimens with extensive fibrosis and fewer parenchymal cells may yield copper concentrations that are nondiagnostic, and therefore the result of hepatic copper quantification should be correlated with the histologic, clinical,

and biochemical data.

Urinary Excretion of Copper

The copper excreted in urine is derived from the free copper circulating in plasma, which represents filterable, non–ceruloplasmin-bound copper. The rate of excretion may exceed 100 µg/24 hours in symptomatic patients. In patients presenting with chronic liver disease, the urinary copper level is elevated above normal levels but may not reach diagnostic levels of more than 100 µg/24 hours. False-positive increases in urinary copper level may be seen in the face of significant proteinuria and urinary loss of ceruloplasmin, and rarely in other liver diseases in which copper storage is increased or in fulminant liver failure. A provocative test for urinary copper excretion with the use of the chelating agent penicillamine has been studied in children but has not been standardized in adults (63). When urinary excretion of copper is tested, it is crucial that a metal-free container be used and that the adequacy of the collection be monitored by correlation with creatinine excretion.

Incorporation of Radioactive Copper into Ceruloplasmin

In patients with Wilson disease and a normal serum level of ceruloplasmin, the incorporation of radioactive copper into ceruloplasmin is significantly reduced in comparison with that in healthy persons or heterozygotes. The failure to incorporate copper into ceruloplasmin within the hepatocyte is seen in all homozygotes with the disease (64). This test has been used to validate the diagnosis of Wilson disease in patients with normal levels of ceruloplasmin. When the test is performed in persons with abnormal levels of ceruloplasmin, as may occur, for example, in generalized hypoproteinemia, the results should be interpreted with caution. This test is now rarely performed because the development of the transjugular liver biopsy technique, as well as the advent of de novo molecular screening, has made it possible to obtain specimens from patients in whom liver biopsy was previously contraindicated. An experimental alternative to radioactive copper is a nonradioactive isotope of copper, ^{65}Cu, which can be detected by mass spectrometric methods (65); however, the utility of this test to distinguish between patients and heterozygous carriers is uncertain.

Molecular Genetic Studies

The identification of the gene for Wilson disease has enabled the molecular genetic diagnosis of this disorder. There are now numerous disease-specific mutations of the gene described; however, the most common mutation is present in only 15% to 30% of most populations (36,37). This makes most patients compound heterozygotes, possessing different mutations on each allele of ATP7B. Direct de novo analysis for the presence of disease-specific mutations is now possible, given the advances in DNA sequencing and screening technology. The ability to establish the diagnosis by this methodology depends on distinguishing disease-specific mutations from polymorphisms of the gene and is further limited by the fact that some of the noncoding regions of the gene, which may also affect gene expression, are not analyzed. It is also possible to screen family members of an affected person by haplotype analysis. This process involves inspecting the patterns of polymorphisms of the DNA in the region surrounding the Wilson disease gene to determine whether mutant regions present in the affected person

have been inherited by family members (30). Future developments in DNA analysis should make it possible to screen for disease-specific mutations in a cost-effective manner, so that de novo population screening will one day prove practical. At present, genetic testing should be used for screening families and used in concert with standard clinical and biochemical testing.

Ultrastructural Studies

In cases in which indeterminate hepatic copper concentrations make it difficult to distinguish between heterozygotes and homozygotes, ultrastructural analysis

for the pathognomonic mitochondrial abnormalities described by Sternlieb (56) may be helpful. The performance of these studies requires proper specimen handling and the assistance of pathologists skilled in electron microscopy, obviously with forethought to process the specimen prospectively for such analysis.

Clinical Manifestations

Patients with symptomatic Wilson disease most frequently present with liver disease or neurologic/psychiatric symptoms. Affected persons detected by family screening are often asymptomatic (also termed *presymptomatic*). The failure to initiate specific treatment for Wilson disease or the disruption of ongoing treatment results in progression to hepatic insufficiency, neuropsychiatric disease, and ultimately hepatic failure and death.

The clinical spectrum of liver diseases associated with Wilson disease is broad (Table 35.3). Younger patients identified by family screening or serial evaluations of isolated biochemical abnormalities are most often asymptomatic. Some patients present with chronic liver disease indistinguishable from other forms of chronic active hepatitis, with or without specific symptoms. In patients with cirrhosis and hepatic insufficiency, ascites, edema, or other stigmata of chronic liver disease, including hepatic encephalopathy, may be observed. When untreated, the liver disease progresses to cirrhosis with hepatic insufficiency, liver failure, and death. Some patients, most often in their second decade of life, present with fulminant hepatitis and an associated nonimmunopathic hemolytic anemia, which without the life-saving intervention of OLT is frequently fatal (see subsequent text). Among patients presenting with Wilsonian fulminant hepatitis, the female-to-male ratio is almost 2:1 (66).

Table 35.3. Clinical Presentations of Wilson Disease

Asymptomatic (presymptomatic)
Hepatic disease
Asymptomatic with only biochemical abnormalities
Chronic active hepatitis
Cirrhosis with hepatic insufficiency and associated signs and symptoms
Fulminant hepatitis with or without hemolytic anemia
Neurologic signs and symptoms
Dystonia with rigidity and contractures
Tremors
Dysarthria and dysphonia

- Gait disturbance
- Choreiform movements

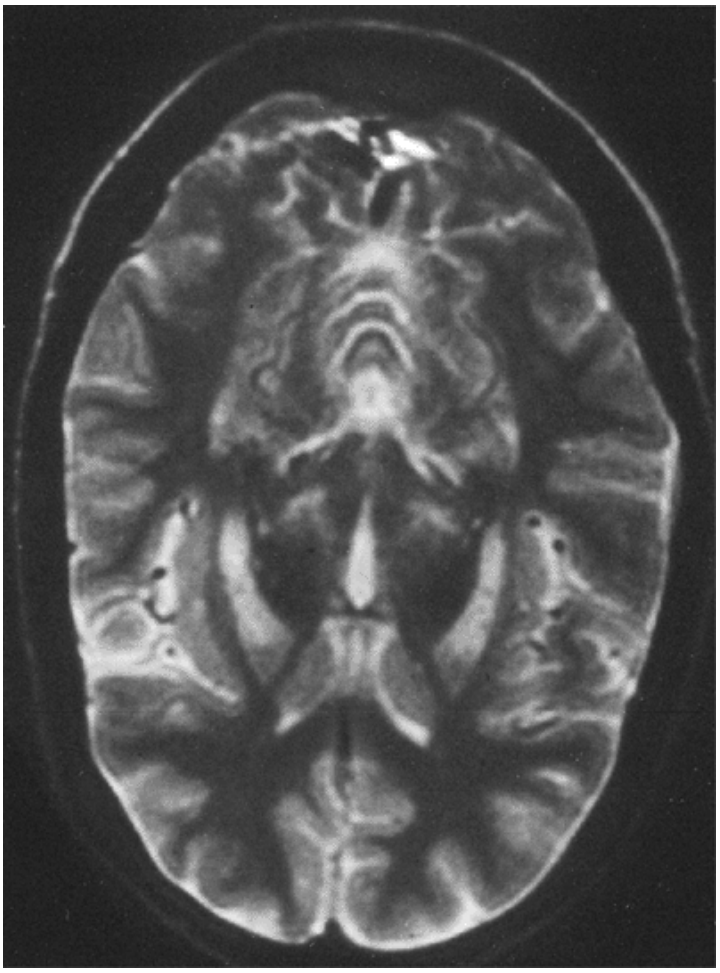
Psychiatric symptoms
- Range from neuroses to psychoses

Renal disease
- Aminoaciduria
- Nephrocalcinosis

The patients in whom the first presenting symptoms of Wilson disease are either neurologic or psychiatric are frequently older than those who present with hepatic symptoms. Most patients with central nervous system involvement are believed to have significant liver disease at the time of presentation; however, hepatic histology is not generally available for these patients because the diagnosis is often established on the basis of a decreased ceruloplasmin level and the presence of K-F rings. Neurologic disease may be manifested as motor abnormalities with parkinsonian characteristics of dystonia, hypertonia and rigidity, chorea or athetosis, tremors, and dysarthria (67). Disabling muscle spasms can lead to contractures, dysarthria and dysphonia, and dysphagia. At this stage of disease, magnetic resonance imaging or computed tomography of the brain may be useful in delineating changes in the basal ganglia (Fig. 35.8).

Wilson disease infrequently presents with abnormalities of other organ systems. Changes induced by copper toxicity in the kidneys include nephrocalcinosis,

hematuria, and aminoaciduria (58) and those in the skeletal system include arthritis, arthralgias, and premature osteoarthrosis (58). Myocardial copper accumulation can cause cardiomyopathy and arrhythmias (68,69), although these are rarely manifested clinically. A more indolent form of hemolytic anemia unassociated with fulminant hepatitis may occasionally be seen (70).



▪ **Figure 35.8** Magnetic resonance image of the brain of a 21-year-old woman with dysarthria, dysphagia, slurred speech, and tremors caused by Wilson disease. Note hyperintensity in the region of the basal ganglia.

Table 35.4. Treatment and Follow-Up Management

Medical therapy for Wilson disease Treatment	Chemical form	Route of administration
British anti-Lewisite	Dimercaptopropanol	Intramuscular
Penicillamine	Dimethylcysteine	Oral
Trientine	Trientine dihydrochloride	Oral

Zinc salts	Zinc sulfate, zinc gluconate, zinc acetate	Oral
Tetrathiomolybdate (experimental)	—	Oral

Maintenance therapy for Wilson disease

Agent	Oral maintenance dose (adult)	Comments	Monitoring of efficacy and compliance
Penicillamine	750–1,000 mg in three to four divided doses	Monitor for lupus-like reactions, marrow suppression; requires supplemental pyridoxine, dose reduction for surgery and pregnancy	Nonceruloplasmin Cu <10 µg/dL Urine Cu >250 µg/24 h
Trientine	750–1,000 mg in three to four divided doses	Monitor for sideroblastic anemia, dose reduction for surgery and pregnancy	Nonceruloplasmin Cu <10 µg/dL Urine Cu >250 µg/24 h
Zinc salts	150 mg in three divided doses	Occasional gastric intolerance	Nonceruloplasmin Cu <10 µg/dL Urine zinc >1,000 µg/24 h Urine Cu <150 µg/24 h

The diagnosis of Wilson disease in the setting of acute fulminant hepatitis deserves special mention because of several unique features. In this setting, fulminant hepatitis is associated with a nonimmunopathic hemolytic anemia and markedly elevated serum and urinary levels of copper. Most of these patients are in the second decade of life, and K-F rings may not yet be apparent. Paradoxically, levels of serum alkaline phosphatase are frequently depressed

(71,72,73), and this feature led to the observation that a ratio of alkaline phosphatase to bilirubin of less than 2 might be diagnostic of Wilsonian fulminant hepatitis (73,74). We and others have observed some patients with Wilsonian fulminant hepatitis in whom this ratio was above 2 (66).

Treatment

The therapeutic options in Wilson disease include pharmacologic treatment and OLT. The aim of medical therapy is to abolish symptoms, if present, and prevent the worsening or progression of disease. Successful therapy may be gauged by clinical improvement or stabilization and by the normalization of biochemical parameters of liver function and copper metabolism. Serial liver biopsies have no role in the management of Wilson disease. Repeated liver biopsies should be performed only to exclude concurrent illness or as part of an experimental treatment protocol. Liver transplantation should be reserved for patients with severe hepatic insufficiency or liver failure occurring in the context of fulminant hepatitis or end-stage liver disease. Transplant recipients subsequently have a normal donor phenotype with respect to copper metabolism, and with rare exception, they do not require further therapy specific to Wilson disease.

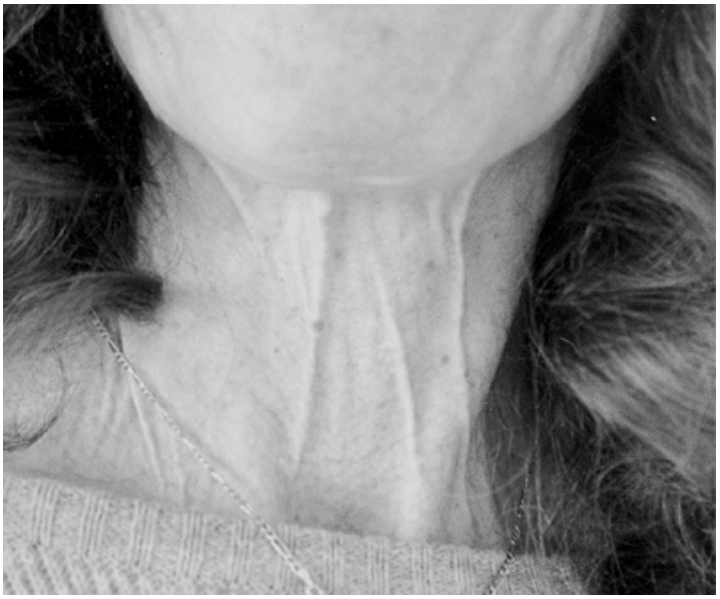
Pharmacologic treatments for Wilson disease include chelating agents and zinc salts (Table 35.4). Chelating agents (e.g., penicillamine, trientine, BAL, and tetrathiomolybdate) remove copper from potentially toxic sites within cells and detoxify the remaining copper. Zinc salts act mainly by blocking the intestinal absorption of dietary copper but also stimulate the biosynthesis of endogenous chelators in the liver, such as metallothioneins, that help detoxify the remaining metal (41).

The treatment of asymptomatic patients and maintenance therapy for previously symptomatic patients are identical (Table 35.4). Patients with hepatic insufficiency or chronic active hepatitis evident only on biochemical testing or histologic examination of the liver should be considered symptomatic and treated with chelation therapy before their medications are changed or the doses reduced for maintenance therapy (see subsequent text). The largest experience for long-term treatment is still with penicillamine, whereas trientine and zinc salts are alternative agents with fewer potential

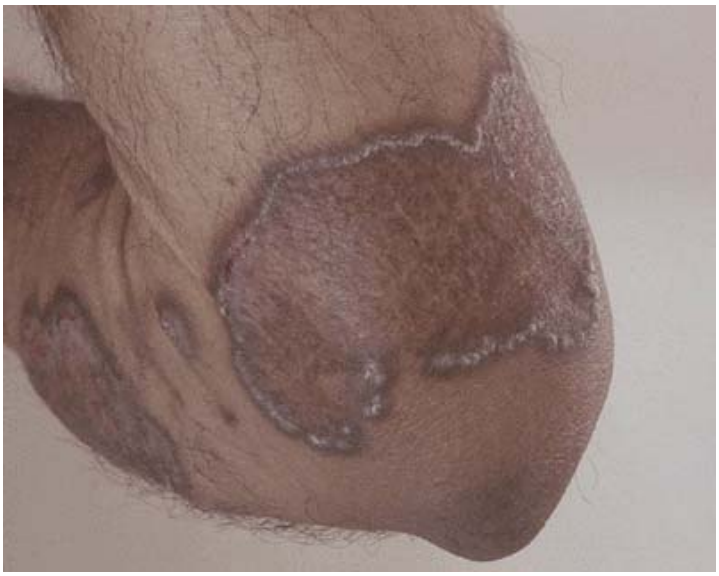
side effects. Both these alternative agents, previously used only for penicillamine-intolerant patients, should now be considered for the initial therapy of asymptomatic patients and for long-term use as maintenance therapy. Regardless of the specific agent chosen, monitoring for efficacy and patient compliance is crucial.

Chelation therapy is indicated as the primary therapy for symptomatic patients with hepatic or neurologic/psychiatric disease. As mentioned in the preceding text, the greatest experience thus far is with penicillamine. The reported incidence of penicillamine-induced side effects varies greatly (58,75), although worsening of neurologic symptoms has been observed in about 10% of symptomatic patients during the early phase of penicillamine therapy. Whether this worsening would have occurred with the use of alternative agents is uncertain and awaits the systematic evaluation of alternative agents as the primary treatment for neurologically affected patients. Late, dermatologic effects of penicillamine include progeric changes in the skin, often visible around the neck (Fig. 35.9), and the cheloid-like lesions of elastosis perforans serpiginosa, which may appear anywhere on the body (Fig. 35.10). Trientine has proved to be

an effective treatment for penicillamine-intolerant patients (76), and experience is growing in the utilization of this agent as a first-line therapy for hepatic and neurologic disease (76,77,78,79). Zinc salts may be used as an alternative initial therapy for patients who cannot tolerate penicillamine or trientine. Although it has been reported that zinc is an effective treatment for symptomatic patients, it may be delayed in its effective onset of action and therefore chelation agents are preferable in this setting. Once clinical and biochemical stabilization has been achieved, typically within 2 to 6 months of the initiation of treatment for most patients but somewhat longer for more severely affected persons, maintenance therapy should be considered. Tetrathiomolybdate, first used to treat animals with copper toxicosis, is currently an experimental agent undergoing evaluation as an initial treatment for patients with neurologic symptoms. Initial reports on the use of tetrathiomolybdate in this setting suggest no worsening of neurologic symptoms and a rapid reduction in circulating non–ceruloplasmin-bound copper during the first 8 weeks of therapy (80). Studies directly comparing the efficacy of this agent with either penicillamine or trientine will help determine its role in the treatment of Wilson disease.



▪ **Figure 35.9** Progeric change (appearance of premature aging) in the skin of the neck, characteristic of patients on long-term penicillamine treatment.



▪ **Figure 35.10** The cheloid-like lesions of elastosis perforans serpiginosa on the elbow of this penicillamine-treated patient may appear on different areas of the body.

BAL, the first available treatment for Wilson disease, is now rarely used, and only as adjunctive therapy for patients with neurologic/psychiatric symptoms refractory to chelation therapy with penicillamine or trientine alone (81). This drug, used in conjunction with oral therapy with penicillamine or trientine, is administered intramuscularly in an oil base. As a lipophilic compound, BAL has the theoretic advantage of possibly crossing the blood–brain barrier more easily. The main drawback of BAL therapy is the difficulty and discomfort involved in administering it and the lack of objective parameters outside clinical evaluation to determine its efficacy.

The dietary consumption of foods with a high copper content should be avoided during the initial phases of treatment. These include organ meats such as liver, in addition to nuts, shellfish, and chocolate. During the maintenance phase of therapy, liberalization of the diet is permitted.

OLT should be considered for patients with Wilsonian fulminant hepatic failure and for those with severe hepatic insufficiency unresponsive to medical therapy (66).

Two different series that retrospectively reviewed the data on OLT for patients with Wilson disease found 1-year survival rates after transplantation to be about 80% (66,82), and more recently, 1-year patient survival was found to be about 87% (83). In this last report, acute renal insufficiency was observed more often in patients who underwent transplantation for fulminant hepatic failure secondary to Wilson disease than in those who underwent transplantation for fulminant hepatic failure of other causes. The acute renal insufficiency resolved without long-term renal damage. During the acute phase of fulminant failure, when toxic copper complexes are being released into the circulation, plasmapheresis, exchange transfusion, and albumin dialysis have been utilized in an effort to further reduce copper-induced toxicity. These interventions may be helpful in

reducing comorbidity and stabilizing the patient, but they have not precluded the need for OLT. Neurologic symptoms may improve after transplantation (66,84). However, it is our opinion that medical therapy should be used for patients with neurologic symptoms in the absence of hepatic failure, especially given the current shortage of donor organs. After OLT in the perioperative period, with rare exceptions, no further specific therapy for Wilson disease is necessary.

Living donor transplantation has been performed for fulminant liver failure secondary to Wilson disease in a few children. Partial grafts from heterozygous parents have been successful, with good organ function in both donor and recipient (85). Currently, living donor transplantation remains limited mostly to children with fulminant hepatic failure, and further study is needed before this procedure can be utilized in adults, for whom a larger portion of the donor liver is required.

The treatment of pregnant women with Wilson disease and persons with Wilson disease who must undergo surgical interventions deserves special mention. The goal of treatment in pregnant patients is to maintain adequate disease control in the mother, reduce her risk for bleeding, and prevent interference with wound healing and the possibility of teratogenicity. Pregnancies have been successful in patients taking penicillamine, trientine, or zinc (86,87,88,89,90). For patients being maintained on chelation therapy, the dosage of penicillamine or trientine should be lowered whenever possible early in the course of the pregnancy. The suggested dosage is 500 mg/day. During the last trimester, the dosage can even be lowered further to 250 mg/day for the 6 weeks through delivery and during wound healing if cesarean section is performed. Zinc therapy can be maintained uninterrupted during pregnancy and postpartum (89).

When patients with Wilson disease maintained on chelating agents must undergo surgery, the dose of their medication should be reduced preoperatively and perioperatively to avoid interference with wound healing. The dosage of penicillamine or trientine can be reduced to 250 to 500 mg daily during this time and rapidly advanced to a maintenance dosage once wound healing has taken place. No adjustment of the dosage is required for patients on zinc therapy, either perioperatively or postoperatively.

We cannot overemphasize that the key to the long-term success of pharmacotherapy for Wilson disease is patient adherence to the use of medications. This can be monitored by history and clinical examinations to detect any changes in the symptoms or signs of liver or neurologic disease, pill counts, screening for biochemical evidence of hepatic dysfunction, measurement of urinary copper or zinc excretion, periodic slit-lamp examinations, and, importantly, biochemical testing for non–ceruloplasmin-bound copper. This last test is the standard by which pharmacotherapeutic doses should be determined and is the single best parameter for gauging the adequacy of treatment. Non–ceruloplasmin-bound copper is a derived number, estimated from the difference between the total serum copper content and the copper content of ceruloplasmin, determined by its oxidase activity (approximately three times the value for ceruloplasmin in milligrams per deciliter). In healthy persons and appropriately treated patients, the value for non–ceruloplasmin-bound copper should be 8 to 12 mg/dL or less. In untreated, inadequately treated, and noncompliant patients, this value is frequently elevated above 25 mg/dL.

The interpretation of the results of urinary copper excretion must take into account the mode of treatment, ability to collect a complete sample, avoidance of

contamination, and appropriate analysis of copper content. During the early phase of treatment with chelating agents, values for urinary copper excretion are frequently greater than 1,000 µg/24 hours. These decline to about 250 to 500 µg/24 hours over time, and despite the continuous use of chelation agents, the values for urinary copper excretion tend to remain at about this level. Values below 250 µg/24 hours suggest noncompliance with therapy, overtreatment, or an incorrect diagnosis from the outset.

The values for 24-hour urinary copper excretion in patients on zinc therapy are not significantly elevated because zinc acts to prevent copper absorption (91). However, a rise in urinary copper excretion to above 150 to 250 µg/24 hours may indicate noncompliance or inadequate therapy, and, if so, they are likely to be accompanied by an increase in non–ceruloplasmin-bound copper level. Urinary and plasma levels of zinc may also be used to monitor compliance with zinc therapy.

The prognosis for patients who comply with pharmacotherapy for Wilson disease is excellent, even if cirrhosis or chronic hepatitis is present at the time of diagnosis (92). Patients with neurologic or psychiatric

symptoms of Wilson disease may continue to recover for months to years after the initiation of treatment. In some patients with neurologic disease or hepatic insufficiency, symptoms or biochemical abnormalities persist but stabilize with treatment. At present, the best way to determine whether and to what extent a patient's disease is reversible is to await a response to treatment.

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Petrukhin K, Fischer SG, Pirastu M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. *Nat Genet*

1993;5:338–343.

*Critical steps in identifying the gene for Wilson disease were localization of the gene to chromosome 13, further sublocalization on this chromosome, and identification of the Menkes disease gene as a copper-transporting adenosine triphosphatase (*25,26,27*). These studies greatly accelerated the search for the Wilson disease gene, which was identified nearly simultaneously and independently by three different laboratories in 1993 (*28,29,30,31*).*

Roberts E, Schilsky ML. A practice guideline on Wilson disease. *Hepatology* 2003;37:1475–1492.

*A review of the evidence-based literature on diagnosing and treating Wilson disease formulated into practical guidelines for the American Association for the Study of Liver Disease (*71*)*

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Original description of Wilson disease as a progressive lenticular degeneration with associated cirrhosis of the liver

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Chapter 36

Hemochromatosis and Iron Storage Disorders

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Key Concepts

- An increase in systemic iron levels is the consequence of (a) inherited excessive intestinal absorption of dietary iron (hereditary hemochromatosis), (b) ineffective erythropoiesis or chronic liver disease, or (c) parenteral iron administration. Excessive intracellular deposition of iron ultimately results in tissue and organ damage.
- Hereditary hemochromatosis (HH) constitutes several inherited disorders characterized by an increased intestinal absorption of iron with its subsequent accumulation in tissues. Most (approximately 90%) patients with HH have mutations in *HFE*, and *HFE*-related HH is one of the most common inherited disorders among whites, with a frequency of about 1 in 250.
- Two independent mutations of the *HFE* gene are principally responsible for *HFE*-related HH. These mutations result in a change of cysteine to tyrosine at amino acid 282 (C282Y) and of histidine to aspartic acid at amino acid 63 (H63D) of the HFE protein. Approximately 95% of persons with *HFE*-related HH are homozygous for the C282Y mutation. Population studies indicate that the penetrance of the C282Y mutation is incomplete, and genetic modifiers may be involved. Some compound heterozygotes with copies of both the C282Y and H63D mutations have a clinically significant degree of iron overload.
- Mutations in the iron-related genes encoding for hemojuvelin, hepcidin, ferroportin, transferrin receptor 2 (TFR2), divalent metal transporter 1 (DMT1), and ferritin result in non–*HFE*-related HH.
- The pathogenesis of nearly all forms of HH involves inappropriately low expression of the iron-regulatory hormone hepcidin, which acts to decrease the export of iron from absorptive enterocytes and reticuloendothelial (RE) cells. Hepcidin is highly expressed in hepatocytes, and it is proposed that HFE protein, TFR2, and hemojuvelin all play a role in the hepatic iron-sensing pathway that regulates hepcidin expression. The C282Y mutation causes functional inactivation of the HFE protein, leading to low hepcidin expression with a resultant increase in duodenal iron absorption.
- In *HFE*-related HH, the excess iron is preferentially deposited in the cytoplasm of parenchymal cells of various organs and tissues, including the liver, pancreas, heart, endocrine glands, skin, and joints. Damage can result in micronodular cirrhosis of the liver and atrophy of the pancreas (primarily islets). Hepatocellular carcinoma, usually in the presence of cirrhosis, is another consequence of excess iron deposition in the liver. Symptoms are related to damage of the

involved organs and include liver failure (from cirrhosis), diabetes mellitus, arthritis, cardiac dysfunction (arrhythmias and failure), and hypogonadotropic hypogonadism.
- The diagnosis of iron overload includes serum iron studies (elevated transferrin saturation [TS], elevated serum ferritin levels), genetic testing, and sometimes liver biopsy to assess the hepatic iron concentration and degree of liver injury. In cases of

HFE-related HH, liver biopsy is usually not indicated if the patient has normal liver enzyme levels and a serum ferritin level below 1,000 ng/mL. Because regular phlebotomy therapy prevents or reverses the accumulation of excess iron and prevents the complications of HH, it is important to identify persons with this inherited disorder early in the disease process.

History

The first medical description of a patient with hemochromatosis was by Trousseau (1) in the French pathology literature in 1865 (Table 36.1). Twenty-four years later, the German pathologist von Recklinghausen (2) was the first to use the term *hemochromatosis*; he thought that the pigmentation ("chrom") in the tissues of patients with the disorder was caused by something circulating in their blood ("hemo"). In 1935, Joseph Sheldon (3), a British geriatrician, published a monograph describing the 311 cases of hemochromatosis that existed in the world literature up to that time. Sheldon concluded that hemochromatosis is an inherited disorder in which tissue injury and damage results from excess iron deposition. He drew accurate conclusions without the techniques of modern molecular medicine available today. The situation was somewhat confused by MacDonald (13), a pathologist at the Boston City Hospital, who believed that hemochromatosis was a nutritional disorder, possibly because he saw many alcoholic patients who happened to be of Irish descent.

It is now known that the prevalence of homozygosity for *HFE*-related hereditary hemochromatosis (HH) is high in the Irish population, approaching 1 in 70 persons (14). In 1976, Marcel Simon et al. (7) definitively showed that classic hemochromatosis is inherited as an autosomal recessive disorder, with linkage to the human leukocyte antigen (HLA) region of the human genome; the gene for hemochromatosis is located on the short arm of chromosome 6. It took another 20 years until the research group at Mercator Genetics successfully identified and cloned the hemochromatosis gene by means of a positional cloning approach using deoxyribonucleic acid (DNA) samples from well-documented patients with hemochromatosis in the United States (9). In 1996, Feder et al. (9) identified *HFE*, a novel major histocompatibility complex (MHC) class I–like gene; homozygosity for a single missense mutation (C282Y) of *HFE* was found in 83% of the patients who were studied. Quickly, several other groups reported their findings in series of patients with hemochromatosis, and homozygosity for the C282Y mutation was found in about 85% to 90% of typical patients (15,16,17,18,19). This discovery has yielded significant benefits in clinical medicine and hepatology, including

more accurate diagnosis of *HFE*-related HH, improved family screening, and evaluation of the role of *HFE* mutations in other liver diseases. Additionally, there has been a wealth of new information about the cellular and molecular mechanisms of iron homeostasis, including the discovery of the iron-regulatory hormone hepcidin. In this chapter, we highlight the new advances in the area of HH and other iron storage disorders, interdigitating these discoveries with the classic pathologic and clinical features seen in these patients.

Table 36.1. Milestones in *HFE*-Related Hereditary Hemochromatosis

Year	Authors (refs.)	Event
~1–300 AD	—	C282Y mutation of *HFE* in Celtic population
1865	Trousseau (1)	First case described

1889	von Recklinghausen (2)	Coined the term *hemochromatosis*
1935	Sheldon (3)	Postulated HH is an inherited defect of iron metabolism
1950	Davis et al. (4,5)	First liver biopsy in HH, and first phlebotomy therapy
1976	Bomford and Williams (6)	Benefit of phlebotomy therapy described
1976	Simon et al. (7)	Linkage to HLA-A
1985	Niederau et al. (8)	Benefit of early diagnosis and therapy
1996	Feder et al. (9)	*HFE* gene cloned, and C282Y and H63D mutations described
1998	Zhou et al. (10)	*HFE* knock-out mouse has iron overload
2001	Nicolas et al. (11)	Hepcidin as iron-regulatory hormone
2003	Bridle et al. (12)	Low hepcidin expression in *HFE*-related HH
1997–present	Many	Cell biology of iron-related proteins

HH, hereditary hemochromatosis; HLA, human leukocyte antigen.

Classification of Iron Overload Syndromes

Many terms have been used in the past to describe HH, such as *idiopathic*, *primary*, and *familial*. The term *hereditary hemochromatosis* should be reserved to describe inherited disorders of iron metabolism that lead to tissue iron loading (Table 36.2). The most common form of this disease, *HFE-related HH*, is caused primarily by homozygosity for the C282Y mutation in the *HFE* gene. However, other heritable forms of iron overload have also been recognized (*non–HFE-related HH*). These include (a) autosomal recessive forms of HH characterized by rapid iron accumulation and caused by mutations in the genes for hemojuvelin and hepcidin (also called *juvenile hemochromatosis*) (20,21), (b) an autosomal dominant form of HH caused by mutations in the ferroportin gene (22,23), (c) an autosomal recessive form of HH resulting from mutations in the gene for transferrin receptor 2 (TFR2) (24,25), and (d) rare forms of HH resulting from mutations in the *divalent metal transporter 1* (*DMT1*) gene (26) or in the regulatory region of ferritin messenger ribonucleic acid (mRNA) (27). Some other types of iron overload may have a heritable component but the genes involved have not yet been identified. For example, *African iron overload* is a familial disorder of iron loading prevalent in sub-Saharan Africa

that is exacerbated by the ingestion of iron-rich home-brewed beer (28,29,30). The degree of iron loading can be similar to that in *HFE*-related HH, but the cellular and lobular distribution of iron is different. In addition, a rare disorder termed *neonatal iron overload* is characterized by increased hepatic iron and severe liver injury present at birth (31,32,33).

Table 36.2. Classification of Iron Overload Syndromes

HEREDITARY HEMOCHROMATOSIS
***HFE*-related**
C282Y/C282Y
C282Y/H63D
Other *HFE* mutations
Non–*HFE*-related
Hemojuvelin (*HJV*) mutations (autosomal recessive)
Hepcidin (*HAMP*) mutations (autosomal recessive)
Ferroportin (*SLC40A1*) mutations (autosomal dominant)
Transferrin receptor 2 (*TFR2*) mutations (autosomal recessive)
Divalent metal transporter 1 (*SLC11A2*) mutations (rare)
Ferritin regulatory mutations (rare)
Miscellaneous
African iron overload
Neonatal iron overload (rare)
SECONDARY IRON OVERLOAD
Anemia caused by ineffective erythropoiesis
Thalassemia major
Sideroblastic anemias
Congenital dyserythropoietic anemias
Congenital atransferrinemia
Aceruloplasminemia
Liver disease
Alcoholic liver disease
Chronic viral hepatitis B and C
Porphyria cutanea tarda
Nonalcoholic steatohepatitis
After portacaval shunt
Miscellaneous
Excessive iron ingestion
PARENTERAL IRON OVERLOAD
Red blood cell transfusions
Iron–dextran injections
Associated with long-term dialysis

Several noninherited syndromes of iron overload are known. In *secondary iron overload*, an underlying disorder causes an increase in iron absorption; examples are disorders of ineffective erythropoiesis and liver disease (34,35). *Parenteral iron overload* is an iatrogenic disorder in which blood transfusions or iron–dextran injections are given to patients who are anemic (36).

In *HFE*-related HH, it has become clear from population studies that not all individuals who have the C282Y/C282Y genotype become iron loaded. This observation indicates that there are many nonexpressing C282Y homozygotes in the population (37,38,39) and has led to a four-category description of *HFE*-related HH: (a) Genetic predisposition with no other abnormality, (b) iron overload (approximately 2 to 5 g) but without symptoms or tissue damage, (c) iron overload with early symptoms (i.e., lethargy, arthralgias), and (d) iron overload with organ damage, particularly cirrhosis.

HFE-Related Hereditary Hemochromatosis

Since the work of Simon et al. in the mid-1970s (7), it has been known that the major gene for HH is located on the short arm of chromosome 6 in the HLA region of the genome. In 1996, investigators at Mercator Genetics used a positional cloning approach to identify *HFE*

as the responsible gene (9). *HFE* codes for a novel MHC class I–like protein that requires interaction with β_2-microglobulin (β_2M) for normal presentation on the cell surface (9). Structural homology with other MHC class I proteins and x-ray crystallographic studies indicate that HFE protein has a large extracellular domain with three α loops, a single transmembrane region, and a short cytoplasmic tail (9,40). In the original work by Feder et al. (9), two missense mutations were identified in *HFE*, one resulting in a change of cysteine to tyrosine at amino acid 282 (C282Y) and the second causing a change of histidine to aspartate at amino acid 63 (H63D). Other *HFE* mutations have been identified, but their frequency appears to be low and their clinical impact is limited. Feder et al. (9) reported that 148 (83%) of 178 patients with typical phenotypic HH were homozygous for the C282Y mutation while 8 (4%) patients were compound heterozygotes, with one allele containing the C282Y mutation and the other allele containing the H63D mutation. These findings were confirmed by subsequent studies that showed that 60% to 100% (mean value, 84%) of patients with typical phenotypic HH were homozygous for C282Y (15,16,17,18,19). Of interest, approximately 6% of patients in these studies, in aggregate, had a clinical syndrome phenotypically similar to that of patients with typical HH but were negative for either *HFE* mutation (19). Some of these patients may have had mutations in known iron-related genes or in as yet unidentified genes involved in iron metabolism.

HFE Gene and Protein

The *HFE* gene is expressed at relatively low levels in most human tissues (9), and Northern blot analysis of lineage-specific human cell lines demonstrates an abundant expression of *HFE* mRNA in cells of epithelial or fibroblastic origin (41). However, unlike other classic MHC class I genes, *HFE* is rarely expressed in lymphopoietic and hematopoietic cells (9). Little is known of the regulation of *HFE* gene expression. In contrast to the expression of genes for other MHC molecules, *HFE* expression is not induced in cultured cells by various cytokines (42). Although in one study of a human intestinal cell line (Caco2) the levels of *HFE* mRNA and protein increased as iron status increased (43), in another study, neither iron chelation nor iron replacement affected *HFE* mRNA levels (44). Sequences known to confer transcriptional regulation by cellular metal ion content have not been identified in the *HFE* gene. Furthermore, sequences homologous to iron-responsive elements (IREs) have not been identified in either the 3′- or 5′-untranslated region of *HFE* mRNA. However, several splice variants of human *HFE* mRNA have been identified. An HFE transcript lacking exons 6 and 7 has been detected (by means of reverse transcription followed by polymerase chain reaction) in human duodenum, spleen, breast, skin, and testicle (45). Because this transcript does not include the sequences encoding the transmembrane and cytoplasmic domains, it is predicted to encode a secretory form of HFE protein. HFE splice variant transcripts lacking exon 2 and/or a portion of exon 4 have been identified in a human liver cell line, a colon carcinoma cell line, and an ovarian cell line (46). Deletion of exon 2 is predicted to eliminate the α_1 loop of HFE protein, whereas deletion of a portion of exon 4 is predicted to affect the α_3 loop. The levels of protein expression and physiologic roles of the splice variant HFE transcripts are not yet known.

The *HFE* gene encodes a 343–amino acid protein consisting of a 22–amino acid signal peptide, a large extracellular domain, a single transmembrane domain, and a short cytoplasmic tail (9). HFE protein is widely expressed in several organs including the liver, placenta, and gastrointestinal tract (i.e., epithelium of esophagus, stomach, duodenum, small intestine, and colon) (47,48,49,50,51). The extracellular domain of HFE protein consists of three loops (α_1, α_2, and α_3), with intramolecular disulfide bonds within the second and third loops. The structure of HFE protein is therefore similar to that of other MHC class I proteins. The two common *HFE* mutations, C282Y and H63D, are in the

extracellular domain. Crystallographic studies demonstrate that the α_1 and α_2 loops of HFE protein form a superdomain consisting of antiparallel β strands topped by two antiparallel α helices (40). The groove between the antiparallel α helices is analogous to the peptide-binding groove in antigen-presenting MHC class I proteins. Several lines of evidence, however, suggest that HFE protein does not participate in antigen presentation. The groove between the α helices in HFE protein is physically narrower than that in antigen-presenting MHC class I molecules, so that the ability to bind peptides is precluded. Indeed, N-terminal sequencing performed on acid eluates from HFE protein found no evidence of peptide binding (40). HFE protein, like other MHC class I molecules, is physically associated with β_2M. This association has been demonstrated in the human duodenum (48) and placenta (47), and in cultured cells (52,53,54).

C282Y mutation of *HFE*

The C282Y mutation results in the substitution of tyrosine for cysteine at amino acid 282 in the α_3 loop and abolishes the disulfide bond in this domain (9). Loss of the disulfide bond was predicted to interfere with the interaction of β_2M with HFE protein (9). Indeed, C282Y mutant protein expressed in cell culture systems demonstrates diminished binding with β_2M and

decreased presentation at the cell surface in comparison with wild-type HFE protein (52,53). The C282Y mutant protein is retained in the endoplasmic reticulum and middle Golgi compartments, fails to undergo late Golgi processing, and is subject to accelerated degradation (53). However, some C282Y mutant protein in patients with HH reaches the cell surface, as detected by immunohistochemistry, although at reduced levels (51). Definitive proof that this mutation can cause HH was provided when knock-in of the C282Y mutation in mice resulted in iron overload (55).

The C282Y mutation is present in most, but not all, patients with a clinical diagnosis of HH. The proportion of patients with HH who are homozygous for C282Y varies in different populations. In the United States, Britain, Australia, Canada, and France, approximately 85% to 90% of patients with a clinical diagnosis of HH are homozygous for C282Y (19), but a lower frequency (60%) has been reported in Italy (56). Conversely, population studies have revealed that the clinical features of HH do not develop in many C282Y homozygotes (37,38,39). This observation suggests incomplete penetrance of the C282Y mutation and raises the possibility that other genes involved in iron homeostasis may act as modifiers of the HH phenotype (57,58,59).

The prevalence of the C282Y mutation is greatest in whites of European ancestry. In this population, the carrier frequency ranges from 10% to 15% (14,19,32,33,34). In other ethnic populations, the C282Y mutation is less common and is always associated with the ancestral white haplotype (60,61). Such studies suggest that the C282Y mutation occurred once on an ancestral (possibly Celtic) haplotype that spread from northern Europe to other regions of the world (62,63). The observation that the haplotype containing the C282Y mutation extends for approximately 7 megabases suggests that the mutation arose during the last 2,000 years (64). It has been proposed that the C282Y mutation, associated with increased iron absorption and the accumulation of body iron stores, provided a selective advantage to a population in which the availability of dietary iron was limited or in which intestinal parasitic infections caused a loss of iron.

H63D mutation of *HFE*

The most common *HFE* mutation in the general population is a missense mutation that results in the substitution of histidine for aspartate at amino acid 63 (H63D) of the HFE protein (9). The H63D mutation is found at a frequency of 15% to 40% in white populations (19), but homozygosity for H63D appears to increase the risk for iron loading only slightly (65). However, the frequency of compound heterozygosity for the H63D and C282Y mutations is greater in patients with iron overload than that predicted for the general population (9,19). It is estimated that the risk for iron loading of the C282Y/H63D compound heterozygote is nearly 200-fold lower than that for the C282Y homozygote (66).

The population distribution of the H63D mutation differs somewhat from that of C282Y. The highest frequencies of H63D are found in European countries bordering the Mediterranean, the Middle East, and the Indian subcontinent (63). The H63D mutation has been found on many haplotypes, which suggests that this less consequential mutation may have arisen historically multiple times and in different populations. Because the haplotype comprising the H63D mutation is shorter (approximately 700 kb) than that of the C282Y mutation, it is thought to be evolutionarily older (64).

Other mutations of *HFE*

HFE mutations other than C282Y and H63D have been identified in isolated patients with iron overload (67,68). These include missense mutations (e.g., S65C, G93R, I105T, and Q127H), splice site mutations (e.g., IVS3+1 G/T and IVS5+1 G/A), frame shift mutations (e.g., V68ΔT, P160ΔC), and nonsense mutations (e.g., R74X, E168X, W169X). Each symptomatic patient carrying one of the missense mutations has carried the C282Y or H63D mutation on the other allele. The two identified splice site mutations cause altered mRNA splicing and exon skipping, resulting in abnormal variants of HFE protein. The frameshift and nonsense mutations result in the production of truncated forms of HFE protein. The relative contribution of *HFE* mutations other than C282Y or H63D to the overall incidence of *HFE*-related HH appears to be small.

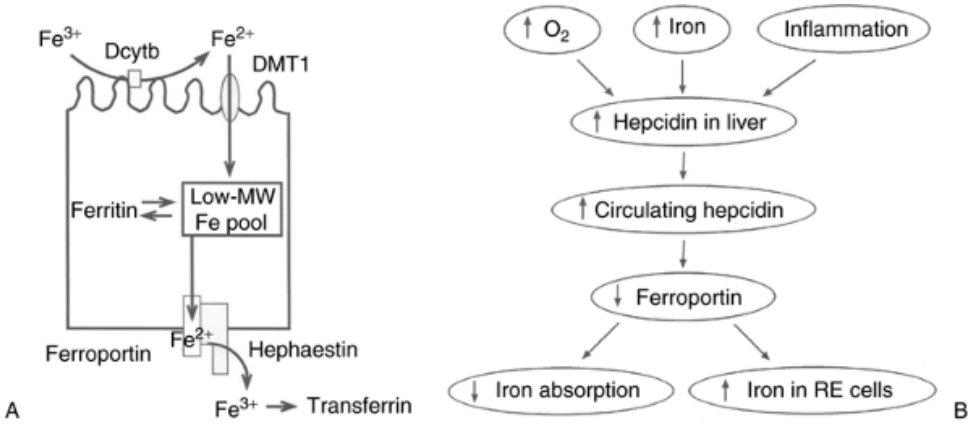
Experimental disruption of the *HFE* gene

Transgenic methodology has provided important information about the functional consequences of *HFE* gene disruption in the whole animal. Four different murine models have been generated: An exon 4 knock-out (10), an exon 3 disruption/exon 4 knock-out (55), an exon 2–3 knock-out (69), and a C282Y knock-in (55). These mice manifest increases in hepatic iron levels (10,55,69), transferrin saturation (TS) (10), and intestinal iron absorption (69). No immunologic consequences of *HFE* disruption have been observed in mice (69). Like patients with *HFE*-related HH, these mice demonstrate relative sparing of iron loading in reticuloendothelial (RE) cells (10,55). Interestingly, iron loading in mice that are homozygous for the C282Y mutation is less severe than that in *HFE* knock-out mice, which indicates that the C282Y mutation is not a null allele (55). Strain differences determine the severity of iron accumulation in *HFE* knock-out mice, supporting the concept that there are genetic modifiers of the HH phenotype (58,70).

Determinants of Duodenal

An increase in intestinal iron absorption is a key characteristic of HH (71,72,73), and, therefore, understanding the pathogenesis of HH requires a review of the determinants of duodenal iron absorption. Because there are no significant physiologic mechanisms to regulate iron loss, iron homeostasis is dependent on tightly linking body iron requirements (approximately 1 mg/day) with intestinal iron absorption. Nearly all absorption of dietary iron occurs in the duodenum, where iron may be taken up either as ionic iron or as heme (73). The absorption of both forms of iron is increased in patients with HH. Uptake of heme occurs by an as yet unidentified transporter. Absorption of ionic iron across the enterocytes occurs in two stages: Uptake across the apical membrane and transfer across the basolateral membrane (Fig. 36.1). Before uptake, ionic iron requires reduction from the ferric to the ferrous state. This is accomplished by the ferric reductases (such as Dcytb), which are expressed on the luminal surface of duodenal enterocytes (74). The ferrous iron crosses the apical membrane using the transporter divalent metal transporter 1 (DMT1) (75,76). Iron taken up by the enterocyte may be stored as ferritin (and excreted in the feces when the senescent enterocyte is sloughed) or transferred across the basolateral membrane to the plasma. This latter process occurs through the transporter ferroportin (77,78,79). The basolateral transfer of iron requires oxidation of iron to the ferric state by the ferroxidase, hephaestin (80). In addition to increased uptake of iron from the diet, patients with *HFE*-related HH demonstrate increased basolateral transfer of iron from the enterocytes to the plasma, and this may be a driving force behind the increased intestinal iron absorption observed in *HFE*-related HH (72). Some studies on

patients with *HFE*-related HH (81,82,83) and *HFE* knock-out mice (84,85) have demonstrated increased expression of mRNAs encoding DMT1 and ferroportin. However, not all investigators have observed upregulation of DMT1 and ferroportin in patients with HH (86,87) or in *HFE* knock-out mice (88,89). In *HFE* knock-out mice, these discrepancies may be due to differences in mouse strain (70) or age (90).



▪ **Figure 36.1** Iron absorption and the role of hepcidin. **A:** Absorption of dietary ionic iron across the duodenal villus enterocyte occurs in two stages: Uptake across the apical membrane and transfer across the basolateral membrane. Before uptake, ionic iron has to be reduced from the ferric to the ferrous state. This is accomplished by the ferric reductases (such as Dcytb), which are expressed on the luminal surface of duodenal enterocytes. The ferrous iron crosses the apical membrane through the divalent metal transporter 1 (DMT1). Iron taken up by the enterocyte is thought to enter a low-molecular-weight (MW) iron pool and then stored as ferritin or transferred across the basolateral membrane to the plasma. This latter process occurs through the transporter ferroportin. The basolateral transfer of iron requires oxidation of iron to the ferric state by the ferroxidase, hephaestin. Ferric iron is then bound to transferrin in the circulation. **B:** Hepcidin expression by the liver is upregulated by increased iron, inflammation, or increased oxygen availability. Circulating hepcidin acts to decrease the functional activity of the iron exporter ferroportin by binding to it and causing its internalization and degradation. In reticuloendothelial (RE) cells, this results in iron sequestration while in duodenal enterocytes it leads to decreased basolateral iron transfer and, therefore, decreased dietary iron absorption. Patients with hereditary hemochromatosis have low hepcidin expression with a consequent increase in iron absorption.

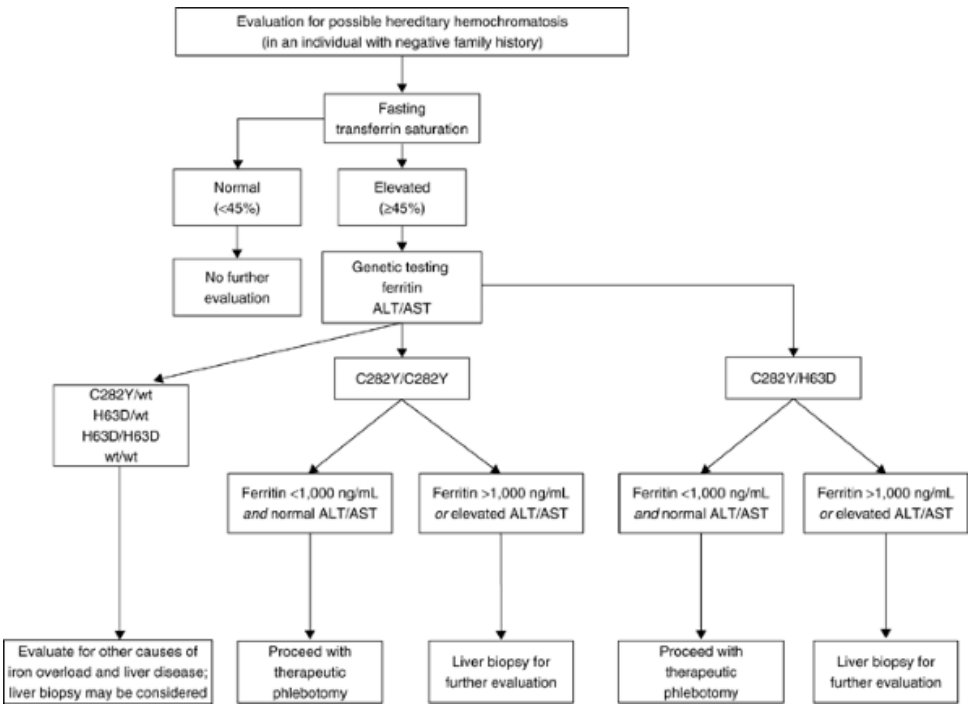
Several biologic factors influence the rate of dietary iron absorption. Reduction in body iron stores, increased erythropoietic activity, decreased blood hemoglobin content, and decreased blood oxygen saturation increase the absorption of iron from the diet (73). In contrast, the presence of systemic inflammation decreases dietary iron absorption. All these factors are thought to act by influencing the levels of hepcidin, an iron-regulatory hormone.

Dysregulation of Hepcidin in Hereditary Hemochromatosis

Hepcidin is a 25–amino acid peptide, first identified in urine and plasma as an antimicrobial peptide (91,92,93). However, its role in influencing systemic iron status has become paramount, and it is now considered to be the

principal iron-regulatory hormone (94,95). The first evidence that hepcidin is involved in iron homeostasis came from the observation that liver hepcidin mRNA expression is

increased in mice with dietary iron loading (93). The fortuitous discovery that knock-out of the hepcidin gene in the mouse led to a HH-like phenotype established the critical role of hepcidin as a negative regulator of intestinal iron absorption (11,96). It was later discovered that hepcidin mutations are responsible for one form of juvenile hemochromatosis (21). Factors regulating intestinal iron absorption (i.e., iron status, erythropoietic activity, hemoglobin levels, oxygen content, and inflammation) also regulate liver hepcidin expression (Fig. 36.2). In each of these situations, intestinal iron absorption varies inversely with liver hepcidin expression. For example, animals with dietary iron overload (93) or systemic inflammation (97) have higher hepatic hepcidin mRNA levels, whereas animals subjected to hypoxia or hemolytic anemia have lower mRNA levels (98,99). Hepcidin is an acute-phase reactant and plays a central role in the hypoferremia of inflammation (i.e., anemia of chronic diseases) (97,100). Hepcidin acts to decrease the functional activity of the iron exporter ferroportin by binding to it and causing its internalization and degradation (101). In the duodenal enterocyte, this leads to decreased basolateral iron transfer and, therefore, decreased dietary iron absorption.



▪ **Figure 36.2** Algorithm for the evaluation of possible hereditary hemochromatosis in a person with a negative family history. ALT, alanine aminotransferase; AST, aspartate aminotransferase; wt, wild type.

Dysregulation of hepcidin expression is thought to play a key role in the pathogenesis of HH. Bridle et al. (12) demonstrated that patients with *HFE*-related HH have low hepatic expression of hepcidin, as do *HFE* knock-out mice, despite excess hepatic iron stores (12,102,103). Overexpression of hepcidin in *HFE* knock-out mice prevents the HH phenotype (104). Although technical problems have hindered the measurement of the mature hepcidin peptide in serum, urinary hepcidin concentrations can be determined. Urinary hepcidin levels are low in patients with HH caused by mutations in *HFE*, *TFR2*, and *HJV* (97,105,106,107,108). Therefore, it is proposed that low circulating levels of hepcidin in these forms of HH cause increased ferroportin-mediated efflux of iron from both RE cells (resulting in iron sparing) and duodenal enterocytes (resulting in increased iron absorption).

The molecular mechanisms by which hepcidin expression is regulated by body iron status

have not been determined. However, it is hypothesized that *TFR2* in hepatocytes may act as an iron "sensor" (24,109). Mutations of *TFR2* cause a rare form of HH in humans (24,25) and *TFR2*-mutant mice have a HH phenotype (110). Despite hepatic iron loading, hepcidin expression is low in patients with *TFR2*-related HH (108) and in *TFR2*-mutant mice (111). This suggests that *TFR2* is necessary for the appropriate transduction of the signal between body iron status and hepcidin expression (24,109). It has been proposed that hepatocytes may modulate hepcidin expression by sensing the circulating levels of diferric transferrin. The concentration of diferric transferrin in portal blood reflects the rate of iron absorption. The binding of diferric transferrin to TFR2 on hepatocytes might transduce a signal that modulates the expression of hepcidin. Likewise, HFE protein and hemojuvelin may participate in this signaling pathway within hepatocytes because inactivating mutations of both these proteins result in low hepcidin expression and iron overload (24,109). HFE protein binds avidly to the classic transferrin receptor 1 (112), but it is not yet clear whether this interaction plays a part in the signaling pathway to hepcidin.

Although the hepatocyte is a strong candidate as the cell type requiring HFE protein for a normal hepcidin response to iron status, some evidence suggests that HFE expression by Kupffer cells may also play a role in the regulation of hepcidin expression. For example, hepatocytes in culture do not respond to changes in iron content with an increase in hepcidin expression (97,113), raising the possibility that another cell type is needed for functional iron sensing. Indeed, *HFE* knock-out mice demonstrate improved iron status after their Kupffer cells are repopulated using transplanted bone marrow from mice with wild-type *HFE* (114).

While it is clear that dysregulation of hepcidin is central to the pathogenesis of *HFE*-related HH, HFE may also be able to influence body iron homeostasis independent of hepcidin. It has been shown that transfection of *HFE* into cultured cells directly influences their iron status (115). Possibly, loss of functional HFE protein in certain cell types (e.g., duodenal crypt cells) could directly contribute to the iron homeostasis abnormalities observed in *HFE*-related HH. Duodenal crypt cells express HFE protein and have been proposed to act as sensors of body iron status through the uptake of plasma diferric transferrin (116). In support of this concept, the duodenal uptake of plasma iron is impaired in *HFE* knock-out mice (117). This observation supports the possibility that functional loss of HFE protein may decrease the iron pool in duodenal crypt cells, resulting in a relatively iron-deficient state in these cells. This could cause increased expression of iron transporter genes in daughter villus enterocytes and lead to increased dietary iron absorption. Although this "crypt cell hypothesis" was proposed to try to explain the excess dietary iron absorption in *HFE*-related HH (116,118), the effect of HFE protein on liver hepcidin expression appears to be paramount.

Non–*HFE*-Related Hereditary Hemochromatosis

Although *HFE* mutations account for the vast majority of HH, other forms of HH have been recognized and are generally grouped together as non–*HFE*-related HH (119) (Table 36.1). Mutations in two different genes, *HJV* and *HAMP*, cause forms of juvenile HH. The *HJV* gene encodes hemojuvelin, a glycosylphosphatidylinositol-anchored protein that has substantial expression in hepatocytes. More than 25 disease-causing mutations have been described in *HJV* (20). Hemojuvelin may be involved in regulating the hepcidin pathway because patients with *HJV*-associated HH (107) and *HJV* knock-out mice (120,121) have low hepcidin expression that may be responsible for increased iron absorption. Inactivating mutations of the *HAMP* gene (that encodes hepcidin) also produce a form of juvenile HH (21).

Two distinct types of ferroportin mutations cause autosomal dominant HH (122,123). The first type of mutation results in ferroportin inactivation, while the second type interferes with the interaction between ferroportin and hepcidin (but ferroportin retains its iron export capability). Inactivating ferroportin mutations cause a cellular distribution of iron loading that differs from that of *HFE*-related HH because iron is retained primarily in RE cells rather than hepatocytes (124). Moreover, TS values tend to be lower than that in *HFE*-related HH. Individuals with the second type of ferroportin mutation fail to respond to

hepcidin and therefore demonstrate a more classical HH phenotype. In both forms of ferroportin-related HH (unlike other types of HH), hepcidin expression is elevated rather than decreased (124).

Mutations in the *TFR2* gene produce an autosomal recessive type of HH that is clinically similar to *HFE*-related HH (24,25). It is not yet known how these uncommon mutations of *TFR2* result in iron overload, but it is possible that they cause abnormal iron sensing by hepatocytes, the predominant site of *TFR2* expression (109).

DMT1 mediates iron uptake at the intestinal brush border and across the membrane of acidified endosomes in cells such as erythyroid precursors (125,126). A single patient with severe hypochromic microcytic anemia and iron overload has been reported to carry

a homozygous mutation (E399D) of DMT1 (26). Cell biology studies suggest that E399D DMT1 protein has a partial loss of function that may limit iron availability to erythyroid precursor cells. The resulting anemia may subsequently stimulate dietary iron absorption, mediated by the partially functional DMT1 in enterocytes (127). This may explain the distinguishing iron overload seen in this patient, along with microcytic anemia.

Ferritin, which is composed of H and L subunits, plays an important role in iron storage and intracellular iron distribution. Synthesis of both ferritin subunits is controlled by an iron-regulatory protein, which binds to the IRE in the 5′-untranslated region of the H- and L-ferritin mRNAs (128). Kato et al. (27) identified a single point mutation (A49U) in the IRE motif of H-ferritin mRNA in four members of a Japanese family affected by dominantly inherited iron overload. When the mutated H-ferritin mRNA is expressed in cultured cells, there is an increase in iron uptake, suggesting that the A49U mutation may be responsible for tissue iron deposition.

Clinical Features of Hereditary Hemochromatosis

In older series of patients with typical phenotypic HH, a number of symptoms and physical findings generally associated with the disorder have been delineated (Tables 36.3, 36.4). Symptoms include fatigue, malaise, abdominal pain, arthralgias, and impotence. Physical findings include hepatomegaly, skin pigmentation, diabetes, and cardiac abnormalities (8,129,130). All physicians should be aware of this constellation of symptoms and findings, but most patients who now come to medical attention have few of these signs or symptoms. More recent series in which patients were identified by family studies, abnormal iron study results on routine screening chemistry panels, or population surveys indicate that most patients are asymptomatic, even on specific questioning about the symptoms of HH (131,132). When symptoms are present, they are the same as those described above, with fatigue and arthralgias being the most common. Therefore, in the face of abnormal iron study results, clinicians should not expect to see the usual symptoms or findings of "classic" HH but should recognize that many C282Y homozygotes are asymptomatic. Alcohol and chronic hepatitis C are potentiating factors in the development of hepatic fibrosis in patients with *HFE*-related HH (133,134,135).

Table 36.3. Symptoms in Patients with Hereditary Hemochromatosis

ASYMPTOMATIC
Abnormal serum iron study results on routine screening chemistry panel
Evaluation of abnormal liver test results
Identified by family screening
Identified by population screening
NONSPECIFIC, SYSTEMIC SYMPTOMS
Weakness
Fatigue
Lethargy

Apathy
Weight loss
SPECIFIC, ORGAN-RELATED SYMPTOMS
Abdominal pain (hepatomegaly)
Arthralgias (arthritis)
Diabetes (pancreas)
Amenorrhea (cirrhosis)
Loss of libido, impotence (pituitary, cirrhosis)
Congestive heart failure (heart)
Arrhythmias (heart)

Table 36.4. Physical Findings in Patients with Hereditary Hemochromatosis

ASYMPTOMATIC
No physical findings
Hepatomegaly
SYMPTOMATIC
Liver
Hepatomegaly
Cutaneous stigmata of chronic liver disease
Splenomegaly
Liver failure: Ascites, encephalopathy
Joints
Arthritis
Joint swelling
Heart
Dilated cardiomyopathy
Congestive heart failure
Skin
Increased pigmentation
Endocrine
Diabetes
Testicular atrophy
Hypogonadism
Hypothyroidism

Diagnosis of Hereditary Hemochromatosis

Once the diagnosis of HH is being considered for a patient after either an evaluation of the symptoms and findings listed in Tables 36.3 and 36.4 or a workup for abnormal results of screening iron studies or a family study, then a definitive diagnosis is relatively

straightforward (133,134,135). The fasting TS (serum iron level divided by transferrin level or total iron-binding capacity, multiplied by 100) and the serum levels of ferritin and liver enzymes should be determined (Table 36.5). It is important that the TS be obtained in the fasting state because the serum iron level varies diurnally, and many breakfast cereals that are highly fortified with iron can raise the serum iron level shortly after ingestion. An elevated TS (≥45%) is recognized as the most common early phenotypic marker of HH; some patients who are homozygous for the C282Y mutation have elevated TS with a normal ferritin level. The sensitivity and specificity of these tests are difficult to determine when young persons are being evaluated (in whom increased iron stores may

not have developed) or when patients have comorbidities, such as chronic liver disease (in which values may be "falsely" elevated). For example, serum ferritin levels are elevated in more than 50% of patients with alcoholic liver disease (136,137), nonalcoholic steatohepatitis (NASH) (138), or chronic viral hepatitis (139,140,141) in the absence of HH. Furthermore, other inflammatory disorders (e.g., inflammatory arthropathies) and various neoplastic disorders (e.g., lymphoproliferative disorders) may cause ferritin levels to rise without any increase in iron stores. Therefore, many results of serum iron studies can be false-positive or false-negative, and reliance on these laboratory values alone may cause significant problems in the diagnosis. The use of *HFE* mutation analysis significantly improves diagnostic accuracy in these challenging patients.

When evaluating the results of *HFE* genotyping obtained from screening family members or from population studies, it is valuable to remember that the C282Y mutation has incomplete penetrance. This is highlighted by the results of two large North American population studies comprising approximately 100,000 and 41,000 primary care patients (38,39). In these two populations, a substantial proportion (27% and 60%, respectively) of female C282Y homozygotes had a TS of less than 45% or 50%, respectively, while the values were smaller (16% and 25%, respectively) for male C282Y homozygotes, with a TS of less than 50%. Similarly, more than 40% of female C282Y homozygotes (43% and 46%, respectively) in these populations had serum ferritin levels in the normal range (<200 ng/mL), while the corresponding values in male C282Y homozygotes were 12% and 24% (serum ferritin levels of <300 ng/mL or 250 ng/mL, respectively). Therefore, for C282Y homozygotes without increases in TS or ferritin level (termed *nonexpressing C282Y homozygotes*), it seems likely that many will not develop a clinically significant degree of iron overload in their lifetimes. Periodic follow-up measurements of serum ferritin levels at about 5-year intervals may be warranted in such individuals, with initiation of phlebotomy therapy if the values rise above normal.

Table 36.5. Diagnostic Criteria for HFE-Related Hereditary Hemochromatosis

Measurements	Normal subjects	Patients with *HFE*-related HH
BLOOD (FASTING)		
Serum iron (µg/dL)	60–180	180–300
Serum transferrin (mg/dL)	220–410	200–300
Transferrin saturation (%)	20–50	45–100
Serum ferritin (ng/mL)		
Men	20–200	150–6,000
Women	15–150	120–6,000
***HFE* MUTATION ANALYSIS**	wt/wt	C282Y/C282Y (homozygote)

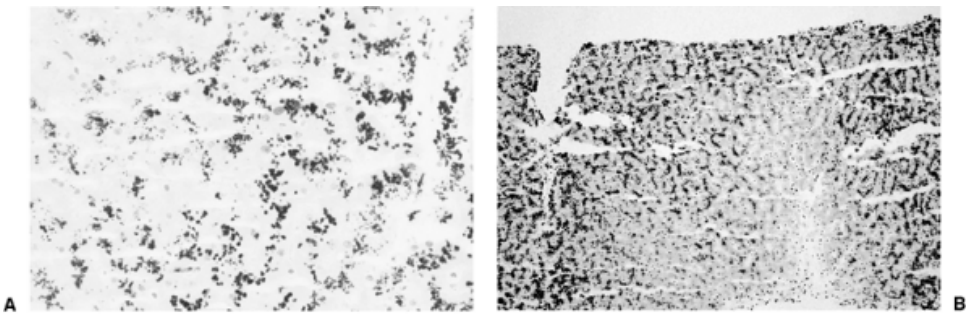
		C282Y/H63D (compound heterozygote)
LIVER Hepatic iron concentration		
µg/g dry weight	300–1,500	1,500–30,000
µmol/g dry weight	5–27	27–550
Liver histology		
Perls' Prussian blue stain	0, 1+	2+ to 4+

wt, wild-type.

In the past, if either a fasting TS or a ferritin level was elevated in an otherwise uncomplicated patient, a liver biopsy was performed to establish or disprove the diagnosis of HH. Perls' Prussian blue staining was used for histochemical iron analysis, and the hepatic iron concentration was measured, with subsequent calculation of the hepatic iron index (HII). In current practice, if a patient has an abnormal result on iron studies, *HFE* mutation analysis is performed; if the patient is found to be a C282Y homozygote or a compound heterozygote (C282Y/H63D), and has normal liver enzymes and a serum ferritin level below 1,000 ng/mL, then a liver biopsy is not required before commencing phlebotomy

treatment. Accordingly, as genetic testing has become more widely available and better understood, fewer liver biopsies are being performed. Four reports from France, United States, and Australia have shown that if the parameters mentioned earlier are used, no patient with significant fibrosis will be missed even if a liver biopsy is not performed (135,142,143,144). An algorithm for the evaluation of possible *HFE*-related HH is shown in Figure 36.2.

On the other hand, if a patient has elevated liver enzymes or a ferritin level above 1,000 ng/mL, a liver biopsy may be performed, and the typical findings of *HFE*-related HH should be recognized. Iron deposition occurs preferentially within the hepatocytes in the periportal region (acinar zone 1) of the hepatic lobule; the gradient of iron deposition decreases toward the pericentral region (acinar zone 3) (Fig. 36.3). With high levels of iron loading, Kupffer cell aggregates (siderotic nodules), iron deposition in bile duct epithelial cells, and increased fibrosis are found in the portal tracts. In other liver diseases associated with increased iron deposition (e.g., alcoholic liver disease, NASH, chronic viral hepatitis), the distribution of iron is usually panlobular, with increased amounts of iron found in sinusoidal lining cells (Kupffer cells) and hepatocytes. The histologic evaluation of iron staining, with a recognition of the pattern seen in *HFE*-related HH as opposed to that associated with secondary iron overload, provides important complementary information to the clinician caring for a patient with liver disease and abnormal results of iron studies. It has been reported that a non-HH pattern of iron distribution reliably predicts the absence of homozygosity for C282Y or the compound heterozygous state (145). Conversely, the HH pattern of iron deposition can be seen in other forms of liver disease in the absence of C282Y homozygosity (145).



▪ **Figure 36.3** Liver biopsy specimen from a patient with *HFE*-related hereditary hemochromatosis stained for storage iron with Perls' Prussian blue stain. The hepatic iron concentration was elevated to 9,840 µg/g dry weight. Iron deposition is found preferentially in hepatocytes (**A**, ×400) in the periportal region (acinar zone 1) of the hepatic lobule, with a decrease in the gradient toward the pericentral region (acinar zone 3) (**B**, ×200). (Courtesy of Elizabeth M. Brunt, MD)

The HII was introduced in a classic paper by the Brisbane group in 1986 (146). It is based on the concept that in patients with HH, the hepatic iron concentration increases progressively with age, whereas in HH heterozygotes or in patients with various forms of liver disease with secondary iron overload, it does not. The HII was originally introduced as a means of differentiating HH homozygotes from both HH heterozygotes and patients with alcoholic liver disease and secondary iron overload, but it quickly came to be used as a surrogate test for HH homozygosity. The HII is calculated by dividing the hepatic iron concentration (in micro moles per gram of liver dry weight) by the patient's age (in years). In several studies, patients with homozygous HH had an HII above 1.9. Now that investigations have been performed with *HFE* mutation analysis used as the "gold standard" for the diagnosis of *HFE*-related HH, it is clear that many patients who are C282Y homozygotes have an HII below 1.9 (143). Therefore, with the availability of genetic testing, the determination of the hepatic iron concentration and HII has little diagnostic value for *HFE*-related HH.

Treatment of Hereditary Hemochromatosis

The treatment of HH remains relatively straightforward (133,134,135). Once the diagnosis has been established by standard iron studies, genetic testing, or liver biopsy, treatment should be initiated with routine therapeutic phlebotomy. Patients should be encouraged to undergo weekly therapeutic phlebotomy with removal of 500 mL (1 U) of whole blood, which represents approximately 200 to 250 mg of iron, depending on the hemoglobin level. Some patients can tolerate the

removal of 2 or even 3 U of blood per week, but 1 U per week is comfortable for most patients. Occasionally, older patients may only be able to tolerate the removal of 0.5 U every other week. Therapeutic phlebotomy should be performed until iron-limited erythropoiesis develops, which is identified by the failure of the hemoglobin level and hematocrit to recover before the next phlebotomy. It is reasonable to monitor the TS and ferritin levels periodically (every 3 months) to predict the return to normal iron stores and encourage patients who are undergoing phlebotomy. Phlebotomy should be continued until the serum ferritin level is less than 50 ng/mL and the TS is less than 50%. It is not necessary for patients to become iron deficient, just depleted of their excess iron stores. Usually, in otherwise uncomplicated patients, each unit of blood removed reduces the serum ferritin level by about 30 ng/mL. This can be a useful way of predicting depletion of

iron stores. Once standard therapeutic phlebotomy has been completed, patients require maintenance phlebotomy. Because in most patients approximately 2 to 3 mg of iron is absorbed per day in excess of their needs, they can be maintained in normal iron balance if 1 U of blood (200 to 250 mg of iron) is removed from them every 3 months.

With treatment, patients generally have an improved sense of well-being, and pain/fullness in the right upper quadrant is reduced (Table 36.6). If liver enzyme abnormalities are present, they will normalize with treatment. Also, if patients require management of diabetes, the need for insulin or oral agents may decrease with successful phlebotomy therapy. On the other hand, testicular atrophy, arthropathy, and established cirrhosis generally are not reversible. Patients with established cirrhosis are still at risk for the development of hepatocellular carcinoma (8,147,148,149), and they should be screened periodically (every 6 months) with abdominal imaging and measurement of α-fetoprotein levels.

Table 36.6. Response to Phlebotomy Therapy in Hereditary Hemochromatosis

- Reduction of tissue iron stores to normal levels
- Improved survival if condition diagnosed and treated before development of cirrhosis and diabetes
- Improved sense of well-being, energy level
- Improved cardiac function
- Improved control of diabetes
- Reduction in abdominal pain
- Decrease in skin pigmentation
- Normalization of elevated liver enzymes
- Reversal of hepatic fibrosis (approximately 30% of cases)
- No reversal of established cirrhosis
- No (or only minimal) improvement in arthropathy
- No reversal of testicular atrophy

Family and Population Screening for Hereditary Hemochromatosis

Once a proband with *HFE*-related HH has been identified, family screening is necessary (133,134,135,150). It is recommended that all first-degree relatives undergo *HFE* mutation analysis. In the past, HLA haplotyping was recommended as a surrogate genetic test, but with the availability of *HFE* mutation analysis, HLA typing is no longer recommended. Both the C282Y and H63D mutations should be analyzed. If a family member is found to be a C282Y homozygote or a compound heterozygote (C282Y/H63D), then therapeutic phlebotomy may be initiated if serum parameters indicate iron overload, such as an elevated ferritin level or TS. Individuals who are C282Y heterozygotes (C282Y/wt), H63D homozygotes (H63D/H63D), or H63D heterozygotes (H63D/wt) are not at risk for progressive iron overload. The issue of screening children by genetic testing raises questions of possible genetic discrimination and stigmatization (150). These issues are not yet resolved at a societal level, but it may be useful to have the spouse of a proband undergo genetic testing to predict the genotype in a child (151). Because C282Y and H63D are such common mutations, occurring in approximately 35% of persons singly or in combination, the chance that the spouse will have a mutation of *HFE* is approximately 1 in 3. If the spouse is homozygous for wild-type *HFE*, then the children do not require genetic testing because they are obligate heterozygotes and are not at increased risk for excess iron storage. In children who are C282Y homozygotes or compound heterozygotes, ferritin levels should be measured yearly and phlebotomy instituted when ferritin levels become elevated.

After the original discovery of *HFE*, it was thought that population screening using genetic testing might be ideal for *HFE*-related HH. Some of the reasons favoring screening are that

C282Y homozygosity is common in white populations, there is a long latent phase before the development of disease manifestations, and treatment is simple and effective (37,38,39,152,153). More than 15 studies have used *HFE* genotyping in population studies, and it has become evident that the C282Y mutation has incomplete penetrance, both in terms of serum iron parameters (i.e., TS, ferritin) and clinical impact (i.e., signs, symptoms, morbidity) (37,38,39,152). Incomplete penetrance of the C282Y mutation raises serious concerns about the cost-effectiveness of large-scale population screening, as does the issue of genetic discrimination about health and life insurance (154,155). Alternative screening approaches such as targeted screening of high-risk groups (e.g., patients with diabetes) and workplace screening may prove efficacious (156,157). At the present time, population

screening requires further evaluation and the most cost-effective methods of early detection of *HFE*-related HH appear to be family screening and evaluation of potential cases by primary care physicians with a high index of clinical suspicion (155,158).

Analysis of *HFE* Mutations in Patients with Liver Disease

Clinicians must frequently determine whether a patient has liver disease with abnormal parameters of iron metabolism or *HFE*-related HH with elevated liver enzymes. Abnormal results of blood iron studies are common in patients with a variety of liver diseases. These are generally associated with hepatocellular types of liver disease rather than the cholestatic syndromes. Approximately 50% of patients with alcoholic liver disease (136,137), NASH (138,159), or chronic viral hepatitis (B and C) (139,140,141) have abnormalities in serum iron parameters. Typically, these abnormalities are limited to an elevated serum ferritin level, but some patients may also have an elevated TS. The hepatic iron concentration is typically normal or slightly elevated, and if the HII is calculated, it is usually normal. *HFE* mutation analysis has been applied to groups of patients with alcoholic liver disease, chronic hepatitis C virus (HCV), NASH, and porphyria cutanea tarda (PCT).

It has been known for more than 20 years that results of iron studies are frequently abnormal in patients with alcoholic liver disease (136,137). Patients with acute alcoholic hepatitis can present with ferritin levels above 1,000 ng/mL and a TS above 100%. These values return to normal with abstinence and recovery from alcoholic hepatitis. Parameters of iron metabolism can also be abnormal in patients with chronic alcoholic liver disease. When *HFE* mutations are examined in patients with chronic alcoholic liver disease, the prevalence of C282Y or H63D is not greater than that in the control population (160,161). Additionally, the absence of a relationship between *HFE* mutations and hepatic iron levels in patients with alcoholic liver disease suggests that the abnormal iron parameters (whether blood studies or hepatic iron concentration) are caused by factors other than *HFE* mutations (160).

A relationship between the hepatic iron concentration and the response to treatment with interferon monotherapy of patients with chronic HCV has been reported (162,163). Studies in the 1990s demonstrated a higher hepatic iron concentration in patients with chronic HCV who did not respond to treatment with interferon monotherapy than in those who did (162,163,164,165). This concept led to the use of therapeutic phlebotomy to deplete iron stores before initial interferon therapy or retreatment with interferon. Iron depletion results in reduced levels of serum alanine aminotransferase (ALT) but does not significantly improve the rate of sustained virologic response (HCV RNA levels undetectable after 6 months without therapy) (166,167,168,169). A recent report indicates that pretreatment hepatic iron concentration is not an independent predictor of response to combined therapy with interferon and ribavirin (170). In a long-term study in patients with HCV who failed to respond to interferon, phlebotomy combined with a low iron diet decreased ALT activity, histologic inflammation and fibrosis, and the hepatic levels of 8-hydroxy-deoxyguanosine (a marker of DNA damage) (171). These results suggest that iron depletion can slow the progression of liver damage in HCV and may reduce the risk for developing hepatocellular carcinoma.

Other studies have suggested that the effect of an increased hepatic iron concentration may be synergistic with that of HCV in causing hepatic fibrosis (172,173,174,175). Studies in which *HFE* mutation analysis was performed in patients with HCV, like those carried out in patients with alcoholic liver disease, showed no difference in the prevalence of C282Y or H63D mutations in comparison with a control population (167,173,174,176,177,178). Some studies have reported that the presence of *HFE* mutations (especially C282Y) in patients with HCV is associated with an increase in fibrosis and cirrhosis (167,173,179), but other studies have not confirmed this association (175,177,178,180). Further investigation is needed to determine the potential role of *HFE* mutations and iron in HCV-induced liver injury and to confirm that long-term iron removal by phlebotomy decreases the progression of hepatic fibrosis and the incidence of hepatocellular carcinoma in patients with HCV.

Results of serum iron studies are often abnormal in patients with NASH (138,159,181,182,183,184) and stainable hepatic iron may also be present. A number of investigations have assessed the prevalence of *HFE* mutations in NASH and the potential role of hepatic iron in disease severity. In several studies, it has been observed that patients with NASH have a higher prevalence of the C282Y mutation (usually heterozygotes) (183,184,185,186), but this is not always the case (187). Not all patients with NASH with elevated hepatic iron levels have the C282Y mutation, indicating that other factors are involved. Some studies also observed an increase in hepatic fibrosis in patients with NASH who carry the C282Y mutation (183,184). While two reports found an association of increased hepatic iron with the development of fibrosis in NASH (183,184), other investigations have not found this association (185,187,188). Regardless of iron levels and *HFE* mutations, several small trials indicate that phlebotomy therapy decreases ALT levels in patients with NASH (189,190,191,192), but the

potential beneficial effects on hepatic histopathology have not been systematically evaluated.

In PCT, the role of abnormal iron stores in disease progression has been known for many years (193,194). In addition, some patients with PCT also have chronic HCV, and alcohol consumption is known to be another risk factor. Studies from the United States, Europe, and Australia have shown that 16% to 49% of patients with PCT have at least one C282Y mutation in *HFE* and 6% to 19% are C282Y homozygotes (195,196,197,198,199,200,201) (Table 36.7). The situation is different for Italian patients with PCT, in whom the prevalence of the C282Y mutation is not increased; rather, the prevalence of the H63D mutation is elevated (202). Therapeutic phlebotomy is beneficial in PCT, inducing a reduction in liver enzyme abnormalities and regression of the skin lesions (193,194). Patients should also be counseled about abstaining from alcohol, and if they have HCV, they should be offered a course of antiviral therapy once their excess iron stores have been depleted.

In summary, all patients with PCT should undergo *HFE* mutation analysis and HCV testing, and phlebotomy therapy is definitely beneficial. For patients with alcoholic liver disease, the role of *HFE* mutation analysis seems to be limited because *HFE* mutations do not appear to contribute to the iron abnormalities seen in these patients. For patients with chronic HCV and NASH, it is reasonable to request *HFE* mutation analysis if the parameters of iron metabolism are abnormal (elevated ferritin level or TS), but the relationships between *HFE* mutations and abnormal iron parameters are not as clear as those in PCT. In patients with HCV or NASH, phlebotomy may be beneficial in decreasing ALT activity and ameliorating liver damage, but additional long-term studies of iron depletion are needed to further assess the possible beneficial effects on the development of cirrhosis and hepatocellular carcinoma.

Table 36.7. Prevalence of *HFE* Mutations And Hepatitis C Virus In Patients With Porphyria Cutanea Tarda

Country (refs.)	No. of patients	Percentage with C282Y/C282Y	Percentage with at least one C282Y mutation	Percentage with at least one H63D mutation	Percentage HCV antibody–positive
United States (195)	70	19	42	31	56
United States (196)	108	19	41	29	59
Britain (197)	41	17	44	32	NR
Sweden (198)	117	17	40	31	NR
Germany (199)	190	12	49	54	15
Australia (200)	27	11	44	44	26
Hungary (201)	50	6	16	36	44
Italy (202)	68	0	3	50	78

HCV, hepatitis C virus; NR, not reported.

Other Iron Storage Disorders

Secondary and Parenteral Iron Overload

Disorders of erythropoiesis and some forms of chronic liver disease can cause increased iron absorption and deposition in tissues (Table 36.2). In these disorders, the iron overload is said to be secondary because the increased absorption of dietary iron is a consequence of an underlying condition (34,35,203,204). A common factor in the iron-loading anemias is refractory anemia, characterized by hypercellular bone marrow and ineffective erythropoiesis. These conditions, which include β-thalassemia and the sideroblastic anemias, can be associated with clinical and pathologic consequences similar to those seen in *HFE*-related HH. In patients with disorders of erythropoiesis who undergo blood transfusions, the iron burden can be increased rapidly by the combined effects of increased iron absorption and the transfusion of iron (in hemoglobin). Therefore, iron toxicity to the liver, heart, and pancreas may develop many years earlier than in patients with *HFE*-related HH (34,205). To minimize iron-induced toxicity in patients with iron-

loading anemias, intensive chelation therapy with deferoxamine is required; this is started at the time when a commitment to long-term transfusion is made (34,35).

Parenteral iron overload is iatrogenic and results from transfusions of red blood cells, injections of iron–dextran, or long-term dialysis (34,35,36). Parenteral iron deposition is initially confined to cells of the RE system, including the Kupffer cells of the liver. When transfusions are required in disorders of erythropoiesis, parenchymal and RE iron overload can coexist. With long-term parenteral iron overload, iron also accumulates in the parenchyma, presumably through the uptake of iron released from RE cells. The degree of structural and functional damage

to the liver and endocrine organs generally parallels the degree of parenchymal iron overload. Sensitive magnetic resonance imaging (MRI) techniques are now available that accurately measure hepatic iron concentration noninvasively (206,207,208), and these may be particularly useful for ongoing assessment of chelation efficacy in patients with disorders of erythropoiesis.

African Iron Overload and Neonatal Iron Overload

The iron overload that occurs in people living in sub-Saharan Africa is now considered to be the result of a non–*HFE*-related genetic trait that can be exacerbated by dietary iron loading (28,29,30). Some persons with African iron overload consume an iron-rich beverage made from fermented maize, but iron overload also occurs in persons who do not drink this beverage. The distribution of accumulated iron in African iron overload is different from that in *HFE*-related HH in that the ratio of the splenic iron concentration to the hepatic iron concentration is higher in African iron overload (28). In addition, iron-loaded Kupffer cells are prominent in African iron overload, whereas the Kupffer cells are relatively spared in *HFE*-related HH. Nevertheless, both African iron overload and *HFE*-related HH can result in portal fibrosis and cirrhosis (28), and the risk for hepatocellular carcinoma is increased (209). Associations of African iron overload with diabetes mellitus, peritonitis, scurvy, and osteoporosis have been described (30). It has been reported that non–*HFE*-related iron overload may occur in African Americans (210,211,212), but further investigations are needed to determine the genetic basis, prevalence, and clinical consequences of this condition. A recent study indicates that approximately 20% of a cohort of Africans and African Americans with iron overload have a novel mutation (Q248H) in the ferroportin gene, suggesting that this mutation may contribute to iron loading (213).

Neonatal or perinatal iron overload is a rare disorder (fewer than 250 cases reported in the literature) in which severe liver disease is associated with parenchymal iron loading, relative sparing of Kupffer cells, and destruction of the normal liver architecture (31,32,33). Most cases have a fatal outcome, and treatment with deferoxamine or an antioxidant cocktail has not been effective (214). Several successful orthotopic liver transplantations have been performed in infants with neonatal iron overload, but long-term survival after this procedure has been disappointing (214). Some cases show evidence of autosomal recessive inheritance, but without mutations in the genes for *HFE*, β_2M, heme oxygenase, transferrin receptor 1, or ferritin (32,215). Neonatal iron overload recurs within sibships at a rate higher than that predicted for simple Mendelian autosomal recessive inheritance, suggesting the role of a maternal factor (32). Immunomodulation with intravenous high-dose immunoglobulin during pregnancy lessens the severity of disease, suggesting that recurrent neonatal iron overload may have an alloimmune component (33).

Summary

With the discovery of *HFE* and other genes involved in iron homeostasis, our understanding of the normal physiology of iron absorption and the pathophysiology associated with mutations of these genes has been dramatically enhanced. *HFE* mutation analysis has greatly improved the ability to diagnose *HFE*-related HH accurately, perform careful family screening, and evaluate patients with liver disease and abnormal results of iron studies.

Interestingly, population studies have revealed that many C282Y homozygotes do not have clinically significant iron overload, suggesting the influence of modifier genes. With a better understanding of the genes that modulate iron homeostasis, the reasons for the phenotypic variability of C282Y homozygotes may be discovered. Mutations in the iron-related genes encoding for hemojuvelin, hepcidin, ferroportin, TFR2, DMT1, and ferritin result in non–*HFE*-related HH. The pathogenesis of nearly all forms of HH involves an inappropriately low expression of hepcidin, an iron-regulatory hormone that acts to decrease the export of iron from duodenal enterocytes and RE cells. As a consequence of this low hepcidin expression, patients with HH having phenotypic expression have increased absorption of dietary iron and elevated TS. Timely phlebotomy treatment of patients with *HFE*-related HH prevents cirrhosis and other iron-induced toxicity.

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Chapter 37

α_1-Antitrypsin Deficiency

David H. Perlmutter

Key Concepts

- Homozygous protease inhibitor phenotype ZZ (PIZZ) α_1-antitrypsin (α_1-AT) deficiency, which has an incidence of 1 in 1,600 to 1 in 2,000 live births, is the most common genetic cause of liver disease in children. It is also associated with chronic liver disease and hepatocellular carcinoma in adults. It is a well-known cause of pulmonary emphysema.
- α_1-AT is an approximately 55-kDa secretory glycoprotein that inhibits destructive neutrophil proteases, elastase, cathepsin G, and proteinase 3. Plasma α_1-AT is predominantly derived from the liver, and its level increases threefold to fivefold during the host response to tissue injury and inflammation. It is the archetype of a family of structurally related circulating serine protease inhibitors termed *serpins*.
- Although emphysema is caused by uninhibited proteolytic destruction of the connective tissue backbone of the lung, liver disease is thought to result from the toxic effects of the mutant α_1-AT molecule retained within the endoplasmic reticulum (ER) of liver cells.
- Screening studies by Sveger in Sweden have shown that only 8% of the PIZZ population have clinically significant liver disease in the first 20 years of life. One series of studies has suggested that a subgroup of PIZZ individuals are predisposed to liver injury because of an inefficient degradation of mutant α_1-ATZ within the ER.
- Altered migration of the abnormal α_1-AT molecule in isoelectric focusing gels is the basis of the diagnosis of α_1-AT deficiency.
- Management of α_1-AT deficiency–associated liver disease is mostly supportive. Liver replacement therapy has been used successfully for severe liver injury.
- Although the clinical efficacy has not been demonstrated, many patients with emphysema due to α_1-AT deficiency are being treated by means of intravenous and intratracheal aerosol administration of purified plasma α_1-AT. An increasing number of patients with severe emphysema are undergoing lung transplantation.

Incidence

The incidence of homozygous protease inhibitor phenotype ZZ (PIZZ) α_1-antitrypsin (α_1-AT) deficiency is highest among people of Scandinavian and northern European descent. This condition is present in approximately 1 in 1,600 to 1 in 2,000 live births (1). Although the incidence of the homozygous deficiency state in North American white populations was originally reported to be 1 in 6,700 live births (2),

more recent studies have demonstrated an incidence almost identical to that in Scandinavian populations (3).

Liver Disease: Clinical Manifestations

Liver involvement is often first noticed at the age of 1 to 2 months because of persistent jaundice. Conjugated bilirubin levels in the blood and serum transaminase levels are mildly to moderately elevated. The liver may be enlarged. Such infants are usually admitted to the hospital with a diagnosis of neonatal hepatitis syndrome and undergo a detailed diagnostic evaluation (4). Infants may also be initially evaluated for α_1-AT deficiency because of an episode of gastrointestinal bleeding, bleeding from the umbilical stump, or bruising (5). A small number of affected infants have hepatosplenomegaly, ascites, and liver synthetic dysfunction in early infancy. An even smaller number have severe fulminant hepatic failure in infancy (6). A few cases are recognized initially because of a cholestatic clinical syndrome characterized by pruritus and hypercholesterolemia. The clinical features among these infants resemble those of extrahepatic biliary atresia, but histologic examination shows a paucity of intrahepatic bile ducts.

Liver disease associated with α_1-AT deficiency may be discovered in late childhood or early adolescence, when the patient is seen with abdominal distension due to hepatosplenomegaly or ascites or with upper intestinal bleeding caused by esophageal variceal hemorrhage. In some of these cases, there is a history of unexplained prolonged obstructive jaundice during the neonatal period. In others, there is no evidence of any previous liver injury, even when the neonatal history is carefully reviewed.

α_1-AT deficiency should be considered in the differential diagnosis for any adult who has chronic hepatitis, cirrhosis, portal hypertension, or hepatocellular carcinoma of unknown origin. An autopsy study in Sweden showed a higher risk of cirrhosis among adults with α_1-AT deficiency than was previously suspected and indicated that α_1-AT deficiency has a strong association with primary liver cancer (7). This study raised the possibility that the risk of clinical liver disease is as high as 25% among men in the fifth and sixth decades of life (Table 37.1).

The only prospective data on the course of α_1-AT deficiency–associated liver injury are from the Swedish nationwide screening study conducted by Sveger (1). In this study, 200,000 newborn infants were screened and 127 PIZZ individuals were identified. Fourteen of the 127 had prolonged obstructive jaundice and 9 of the 14 had severe liver disease, as indicated by clinical and laboratory criteria. Another 8 of the 127 PIZZ infants had mildly abnormal serum bilirubin or serum transaminase levels or hepatomegaly. Approximately 50% of the rest of the 127 only had abnormal transaminase levels (8). Published results of follow-up studies of the original cohort of 127 PIZZ children at 18 years of age (9) show that more than 85% had persistently normal serum transaminase levels with no evidence of liver dysfunction. At 26 years of age, only 10% of the original population had evidence of elevated serum transaminase levels (Sveger T., personal communication, 2001). Issues not addressed by the Sveger study are whether 18-year-olds with α_1-AT deficiency have persistent subclinical histologic abnormalities, despite a lack of clinical or biochemical evidence of liver injury, and whether liver disease will eventually become clinically evident during adulthood.

Table 37.1. Liver Disease Associated With α_1-Antitrypsin Deficiency

CLINICAL FEATURES

- Prolonged jaundice in an infant
- Neonatal hepatitis syndrome
- Mild elevation of transaminase levels in a toddler
- Portal hypertension in a child or adolescent
- Severe liver dysfunction in a child or adolescent
- Chronic hepatitis in an adult
- Cryptogenic cirrhosis in an adult
- Hepatocellular carcinoma in an adult

DIAGNOSTIC FEATURES

- Diminished serum levels of α_1-antitrypsin
- Abnormal mobility of α_1-antitrypsin in isoelectric focusing (PIZ)
- Periodic acid–Schiff–positive, diastase-resistant globules in liver cells

It is still not clear what clinical manifestations or abnormal laboratory test results can be used to predict a poor prognosis for patients with α_1-AT deficiency–associated liver disease. Results of one study suggested that persistence of hyperbilirubinemia, hard hepatomegaly, early development of splenomegaly, and progressive prolongation of prothrombin time were indicators of poor prognosis (10). In another study, elevated transaminase levels, prolonged prothrombin time, and a lower trypsin inhibitor capacity correlated with a worse prognosis (11). However, the author and his colleagues have found that some children with α_1-AT deficiency–associated liver disease can lead relatively normal lives for years after the development of hepatosplenomegaly and mild prolongation of prothrombin time. In a review of 44 patients with α_1-AT deficiency seen in the specialty practice at St. Louis Children's Hospital, 17 patients had cirrhosis, portal hypertension, or both (12). Nine of the 17 patients with cirrhosis or portal hypertension had a prolonged, relatively uneventful course for at least 4 years after the diagnosis of cirrhosis or portal hypertension. Two of these patients eventually underwent liver transplantation, but seven were leading relatively healthy lives for as long as 23 years after being diagnosed as having severe α_1-AT deficiency–associated

liver disease. Patients with the prolonged stable course could be differentiated from those with a rapidly progressive course on the basis of overall life functioning but not on the basis of other more conventional clinical or biochemical criteria. Therefore, prediction of poor prognosis for α_1-AT deficiency–associated liver disease and for the timing of liver transplantation depends more on the overall functioning of the child than on the histologic findings or laboratory data.

There is currently no evidence that the heterozygous α_1-AT phenotype causes liver disease in children by itself. It is not clear whether heterozygous adults are predisposed to liver injury. Early studies of liver biopsy collections suggested that there was a relation between heterozygosity and the development of liver disease (13). A retrospective study at the Mayo Clinic showed a higher prevalence of heterozygosity for α_1-ATZ in liver transplant recipients than in the general population, including a group of patients without another explanation for liver disease (14). However, both these studies were biased in ascertainment and did not include concurrent prospective controls. Results of a cross-sectional study of patients with α_1-AT deficiency in a referral-based Austrian university hospital, who were reexamined with the most sophisticated and sensitive assays available, suggested that liver disease in heterozygotes can be accounted for, to a great extent, by infections with hepatitis B or C virus or by autoimmune disease (15).

Although the foregoing findings taken together give a strong impression that heterozygotes for α_1-ATZ are susceptible to liver disease, the literature does not provide convincing evidence that liver injury can be explained by the α_1-AT heterozygous state alone.

Liver disease has been described for several other allelic variants of α_1-AT. Children with compound heterozygosity type PISZ are affected by liver injury in a manner similar to that of PIZZ children (1,8,9). There are several reports of liver disease in α_1-AT deficiency–type PIMMalton (16,17). This is a particularly interesting association because the abnormal PIMMalton α_1-AT molecule has been shown to undergo polymerization and retention within the endoplasmic reticulum (ER) (17). Liver disease has been detected in single patients with several other α_1-AT allelic variants, such as PIMDuarte (18), PIW (19), and PIFZ (20), but it is not clear whether other causes of liver injury for which there are more sophisticated diagnostic assays, such as infection with hepatitis C and autoimmune hepatitis, have been completely excluded in these cases.

Lung Disease: Clinical Manifestations

The association between α_1-AT deficiency and the premature development of pulmonary emphysema is well documented (21). Cigarette smoking markedly accelerates this destructive lung disease, reduces the quality of life, and markedly shortens the longevity of these persons (22). There is still, however, wide variability in the incidence and severity of destructive lung disease within the α_1-AT–deficient population (23). There are even PIZZ individuals who smoke but do not have any symptoms of lung disease or pulmonary function abnormalities or do not experience them until the seventh or eighth decade of life. Although results of one study have suggested the possibility that a subtle degree of hyperinflation can be detected with pulmonary function testing in infants with α_1-AT deficiency (24), results of another study did not show any significant difference between the pulmonary function of PIZZ individuals aged 13 to 17 and that of an age-matched control group (25). True clinical symptoms of α_1-AT deficiency–associated emphysema do not begin until the third decade of life. The usual initial symptoms are shortness of breath, wheezing, cough, sputum production, and frequent chest infections (26).

There is still limited information about the incidence of liver disease among persons with α_1-AT deficiency and emphysema. In one recent study of 22 PIZZ patients with emphysema, there was an elevated transaminase level in 10 patients, and cholestasis was present in 1 patient (27). Liver biopsies were not performed in this study and may be necessary for the accurate determination of the extent of liver injury in these patients.

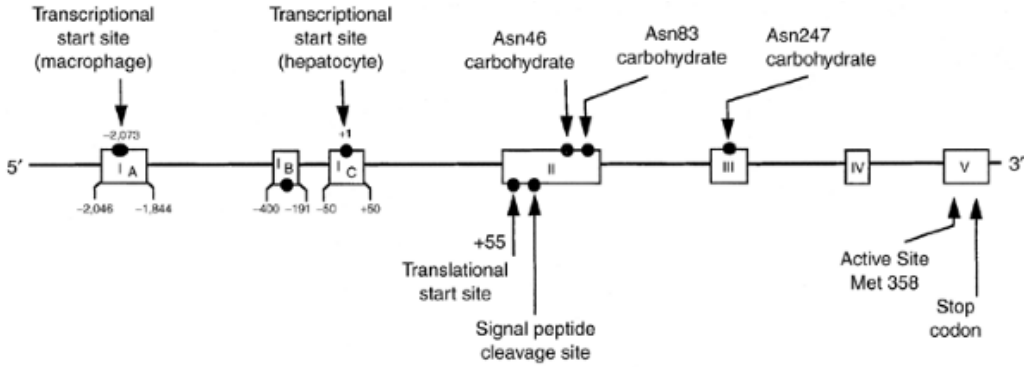
α_1-Antitrypsin Structure, Function, and Physiology

α_1-Antitrypsin Gene Structure

α_1-AT is encoded by a 12.2-kb gene on chromosome 14q31-32.3 (28). There is a sequence-related gene approximately 12 kb downstream. Because there is no evidence that it is expressed, the downstream gene is considered a pseudogene.

The gene (Fig. 37.1) is composed of five exons and four introns (29). Exon I_C, the 5′ portion of exon II, and the 3′ portion of exon V are noncoding regions. The first intron is 5.3 kb long, contains a short open reading frame, an Alu family sequence, and a pseudotranscription initiation codon. Apparently, the short open reading frame does not code for protein. The α_1-AT messenger ribonucleic acid (mRNA) expressed in the liver is 1.4 kb long (30). In macrophages, the α_1-AT mRNA is slightly longer (30). There are three forms of α_1-AT mRNA in macrophages, depending on

transcription initiation sites in two upstream exonic structures (exons I_A and I_B) (30, 31).



▪ **Figure 37.1** Schematic representation of the structure of the α_1-antitrypsin gene (*not to scale*).

α_1-Antitrypsin Protein Structure

α_1-AT is a single-chain, approximately 55-kDa polypeptide with 394 amino acids and three asparagine-linked complex carbohydrate side chains (32). There are two major serum isoforms depending on the presence of a biantennary or triantennary configuration of the carbohydrate side chains. α_1-AT is the archetype of a family of structurally related proteins called *serpins* (serine protease inhibitors), which includes antithrombin III, α_1-antichymotrypsin, C1 inhibitor, α_2-antiplasmin, protein C inhibitor, heparin cofactor II, plasminogen activator inhibitors I and II, and protease nexin I (33). A serpin-like structure is also found in several cellular proteins, trophic factors, and circulating carrier proteins, such as corticosteroid- and thyroid hormone–binding globulin.

Many studies of the structural characteristics of α_1-AT have shown that it is essentially composed of two central β sheets surrounded by a small β sheet and nine α-helices (33). The dominant structure is the five-stranded, β-pleated sheet called the *A sheet* (Fig. 37.2). A mobile reactive center loop rises above a gap in the center of the A sheet (34,35,36).

The Protease Inhibitor System for Classification of Structural Variants of α_1-Antitrypsin

Variants of α_1-AT in humans are classified according to the PI phenotype system, as defined by agarose electrophoresis or isoelectric focusing of plasma in polyacrylamide at acid pH (37). The PI classification system assigns a letter to variants according to migration of the major isoform. For example, the most common normal variant migrates to an intermediate isoelectric point, designated M. Persons with the most common severe deficiency have an α_1-AT allelic variant that migrates to a high isoelectric point, designated Z (Fig. 37.3). Even greater polymorphic variation of α_1-AT has been detected by means of restriction fragment length and direct deoxyribonucleic acid (DNA) sequence analysis. With these techniques, in addition to isoelectric focusing, investigators have identified more than 100 allelic

variants (38).

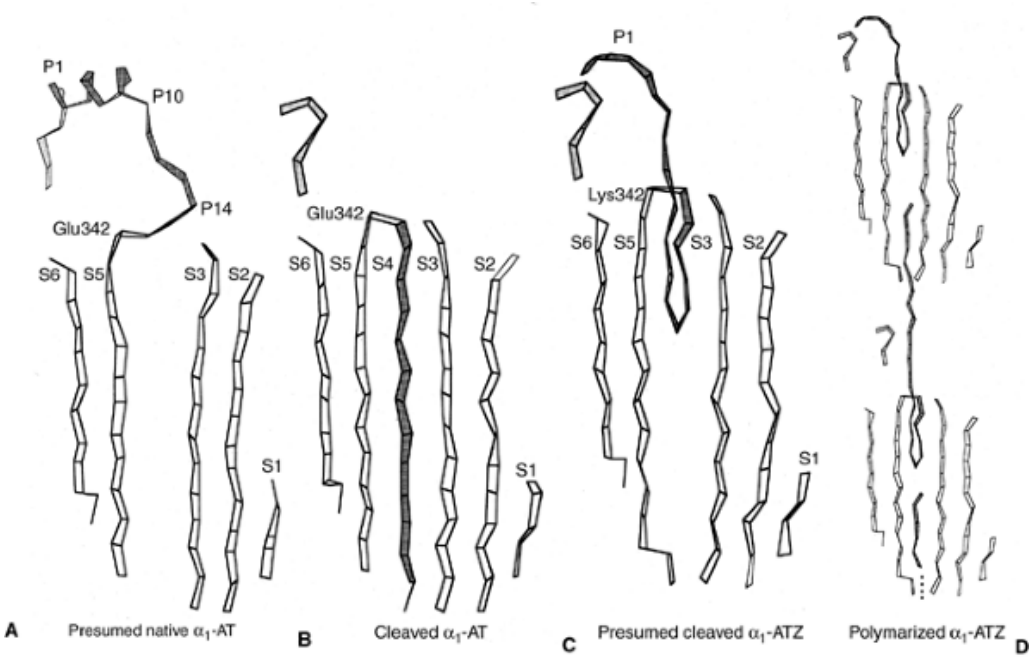
Normal allelic variants

The most common normal variant of α_1-AT is termed *M1* and is found in 65% to 70% of whites in the United States (2). There are many rare normal allelic variants with allelic frequencies of less than 0.1%. For each of these variants, serum concentration and functional activity of α_1-AT are within the normal range.

Null allelic variants

α_1-AT variants in which α_1-AT is not detectable in serum are called *null allelic variants* (Table 37.2). The inheritance of a null allelic variant with another null variant or a deficiency variant is associated with premature development of emphysema (39,40). There is no evidence for liver injury in persons with null variants who were examined in detail (41). Potential molecular mechanisms for the null phenotype have been identified by DNA sequence analysis of a number of null variants (38,39,40,41,42,43,44). They include deletion of all α_1-AT coding exons, substitutions that result in stop codons, frameshift mutations, and single base substitutions. In at least three cases, the frameshift mutation resulted in an abnormal truncated protein that is retained in the ER ($null_{Hong\ Kong}$, $null_{Clayton}$, $null_{Saarbrucken}$) (41,42,43,45). Detailed study of some of these variants has raised questions about the classification. Although α_1-AT was not detected in the serum of a patient with what has been called α_1-AT $null_{Ludwigshafen}$ (44), this mutant molecule is synthesized and secreted in transfected heterologous cells (46). Its rate of secretion is slightly decreased and

the secreted mutant protein lacks functional activity. It is not yet known whether instability or accelerated catabolism in vivo is the explanation for the inability to detect this mutant α_1-AT molecule in serum specimens.



▪ **Figure 37.2** Ribbon diagrams of the A sheet and reactive-site loop of α_1-antitrypsin (α_1-AT) in several different states. **A:** Presumed native α_1-AT. This state is presumed because it has not been crystallized; however, it is generated

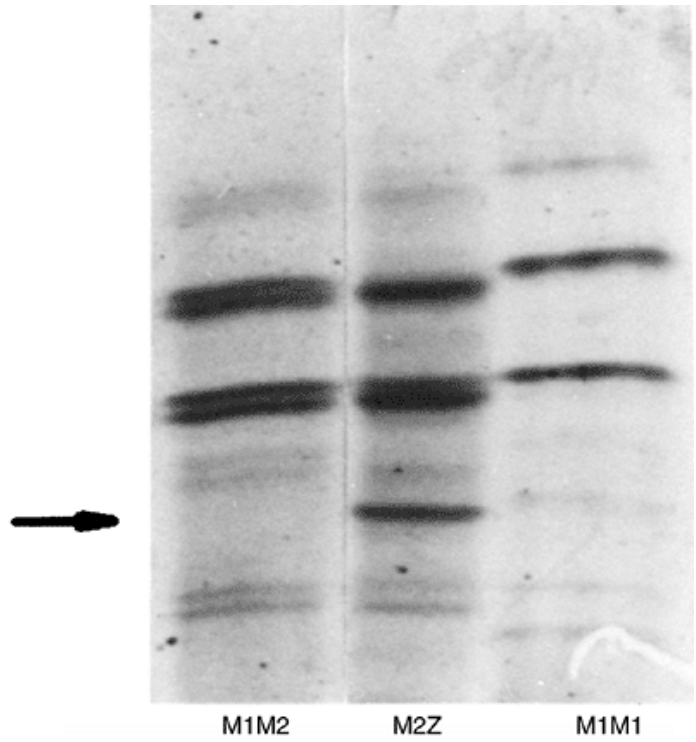
by computer models on the basis of the crystal structures of cleaved α_1-AT and native ovalbumin. The reactive-site loop is shown as a densely dotted ribbon. The β-helices of the A sheet are shown in white and are referred to as S1 to S6. The position of glutamate 342 is indicated. There is a gap in the A sheet between S3 and S5. **B:** Cleaved α_1-AT. The reactive-site loop is cleaved. It inserts into the A sheet and is now referred to as S4. **C:** Presumed cleaved α_1-ATZ. The reactive-site loop spontaneously collapses into the gap in the A sheet, but because of the substitution of lysine at residue 342, it cannot fully insert. **D:** Polymerized α_1-ATZ. The gap in the A sheet of the upper α_1-ATZ molecule is now filled with the reactive-site loop of the lower α_1-ATZ molecule. The gap in the A sheet of the lower α_1-ATZ molecule is filled with the reactive-site loop of another α_1-ATZ molecule represented by a *dotted line*. (Adapted from Carrell RW, Evans DL, Stein DE. Mobile reactive centre of serpins and the control of thrombosis. *Nature* 1991;353:576and Lomas DA, Evans DL, Finch JT, et al. The mechanism of Z α1-antitrypsin accumulation in the liver. *Nature* 1992;357:605, with permission.)

Dysfunctional variants

α_1-$AT_{Pittsburgh}$ is the most well-characterized dysfunctional variant (47), which was identified in a 14-year-old boy who died of an episodic bleeding disorder. A single–amino acid substitution, Met to Arg at residue 358, converted α_1-AT from an elastase inhibitor to a thrombin inhibitor. The episodic nature of the illness was attributed to changes in the synthesis of the mutant protein during the host response to acute inflammation and tissue injury, the acute-phase response. The α_1-AT $M_{Mineral\ Springs}$ (46,48) and $null_{Ludwigshafen}$ (44,46) probably are other examples of dysfunctional variants.

Deficiency variants

Several variants of α_1-AT that are associated with a reduction in serum concentrations have been described and are called *deficiency variants* (Table 37.3). Some of these variants are not associated with clinical disease, such as the S variant (29,49). Other deficiency variants are associated only with emphysema, such as $M_{Heerlen}$ (50), $M_{Procida}$(51), and P_{Lowell} (52). In two persons with M_{Malton} and one with M_{Duarte}, hepatocyte α_1-AT inclusions and liver disease have been found with emphysema (16,17,18). In one person with the α_1-AT S_{Iiyama} variant, emphysema and hepatocyte inclusions were reported, but this patient did not have liver disease (53). The most common deficiency variant, the Z variant, is associated with emphysema and liver disease, as discussed later.



▪ **Figure 37.3** Isoelectric focusing of human serum samples for diagnosis of α_1-antitrypsin deficiency. Serum samples from a person with the normal M1M2 variant, from an M2Z heterozygote, and from a person with the normal M1M1 variant were subjected to isoelectric focusing with the anode at top and cathode at bottom. Migration of the Z allele is indicated by the *arrow.* (Gel provided by JA Pierce, St. Louis, MO. From Perlmutter DH. Alpha-1-antitrypsin deficiency. In: Snape WJ, ed. *Consultations in gastroenterology*. Philadelphia: WB Saunders; 1996:793, with permission.)

Function

α_1-AT is an inhibitor of serine proteases in general, but its most important targets are neutrophil elastase, cathepsin G, and proteinase 3, proteases released by activated neutrophils. Several lines of evidence suggest that inhibition of neutrophil elastase is the major physiologic role of α_1-AT. First, persons with α_1-AT deficiency are susceptible to premature development of emphysema, a lesion that can be induced in experimental animals by means of instillation of excessive amounts of neutrophil elastase (54). These observations have led to the concept that destructive lung disease may result from perturbations of the net balance of elastase and α_1-AT within the local environment of the lung (55). Second, the kinetics of association for α_1-AT and neutrophil elastase are more favorable, by several orders of magnitude, than those for α_1-AT and any other serine protease (56). Third, α_1-AT constitutes more than 90% of the neutrophil elastase inhibitory activity in the one body fluid that has been examined—pulmonary alveolar lavage fluid (55).

α_1-AT acts competitively by allowing its target enzymes to bind directly to a substrate-like region within its reactive center loop. The reaction between enzyme and inhibitor is essentially of second order, and the resulting complex contains one molecule of each of the reactants. A reactive-site peptide bond within the inhibitor is

hydrolyzed during the formation of the enzyme–inhibitor complex. The complex of α_1-AT and serine protease is a covalently stabilized structure resistant to dissociation by denaturing compounds, including sodium dodecyl sulfate and urea. The interaction between α_1-AT and serine protease is suicidal in that the modified inhibitor is no longer able to bind with or inactivate the enzyme. There is also a profound alteration in the structure of the enzyme, including disruption of the catalytic site, such that the enzyme becomes inactive and subject to proteolytic destruction (57). Studies have shown that the irreversible trapping of target enzyme is mediated by a profound conformational change in α_1-AT, such that the cleaved reactive-loop binding enzyme inserts into the gap in the A sheet (58). Carrell and Lomas (58) likened the inhibitory mechanism to a mousetrap: The active inhibitor circulates in the metastable, stressed form and then springs into the stable, relaxed form to lock the complex with its target protease.

The net functional activity of α_1-AT in complex biologic fluids may be modified by several factors. First, the reactive-site methionine may be oxidized and thereby rendered inactive as an elastase inhibitor (59). In vitro, α_1-AT is oxidatively inactivated by oxidants released by activated neutrophils and alveolar macrophages of cigarette smokers (60,61). Second, the functional activity of α_1-AT may be modified by proteolytic inactivation. Several members of the metalloproteinase family, including collagenase and *Pseudomonas* elastase, and the thiol protease family can cleave and inactivate α_1-AT (62). Studies have shown that the pathogenesis of bullous pemphigoid may involve uninhibited neutrophil elastase activity at the dermal–epidermal junction because α_1-AT is cleaved and inactivated by matrix metalloproteinase-9 (MMP-9)–gelatinase B in the skin (63,64). Third, DNA, which is often released from neutrophils at sites of inflammatory activation and phagocytosis, can impair the cathepsin G inhibitory activity of α_1-AT (65).

Although α_1-AT from the plasma or liver of persons with PIZZ α_1-AT deficiency is functionally active (66), there may be a decrease in its specific elastase inhibitory capacity. Ogushi et al. (67) showed that the kinetics of association with neutrophil elastase and the stability of complexes with neutrophil elastase were significantly decreased for α_1-AT isolated from PIZZ plasma. There was no decrease in the functional activity of α_1-AT in individuals homozygous for the S variant.

Results of several studies have indicated that α_1-AT protects experimental animals from the lethal effects of tumor necrosis factor (68,69). Most of the evidence from these studies indicates that this protective effect

is due to inhibition of the synthesis and release of platelet-activating factor from neutrophils (69,70), presumably through the inhibition of neutrophil-derived proteases. The antiapoptotic effect of α_1-AT on vascular smooth muscle cells (71) also probably involves the inhibition of extracellular matrix degradation by neutrophil-derived proteases.

Table 37.2. Null Variants of α_1-Antitrypsin

Variant	Defect	Site	Clinical disease: Liver	Clinical disease: Lung	Cellular defect
$Null_{Granite\ Falls}$	Single base deletion	Tyr160	-	+	No detectable

					RNA
$Null_{Bellingham}$	Single base deletion	Lys217	-	+	No detectable RNA
$Null_{Mattawa}$	Single base insertion	Phe353	-	+	?IC degradation
$Null_{Hong\ Kong}$	Dinucleotide deletion	Leu 318	-	+	IC accumulation
$Null_{Ludwigshafen}$	Single base substitution	Isoleu92–Asp	-		?Accelerated catabolism
$Null_{Clayton}$	Single base insertion	Glu363	-	+	IC accumulation
$Null_{Bolton}$	Single base deletion	Glu363	-	+	?IC degradation
$Null_{Isola\ di\ Procida}$	Deletion	Exons II–V	-	+	Unknown
$Null_{Riedenburg}$	Deletion	Exons II–V	-	+	Unknown
$Null_{Newport}$	Single base substitution	Gly115–Ser	-	+	Unknown
$Null_{Bonny\ Blue}$	Intron deletion		-	+	Unknown
$Null_{New\ Hope}$	Two base substitutions	Gly320–Glu Glu342–Lys	-	+	Unknown
$Null_{Trastavere}$	Single base substitution	Trp194–stop	-	+	Unknown
$Null_{Kowloon}$	Single base substitution	Tyr38–stop	-	+	Unknown

$Null_{Saarbruecken}$	Single base insertion	Pro362–stop	-	+	IC accumulation
$Null_{Lisbon}$	Single base substitution	Thr68–Ile	-	+	Unknown
$Null_{West}$	Intron deletion	—	-	+	Unknown

RNA, ribonucleic acid; IC, intracellular; ?, not proven.

Results of several studies indicate that α_1-AT has functional activities other than the inhibition of serine protease. The carboxyl-terminal fragment of α_1-AT, which can be generated during the formation of a complex with serine protease or during proteolytic inactivation by thiolproteinases or metalloproteinases, is a potent neutrophil chemoattractant (72). The chemotactic response is equivalent to that elicited by formyl-methionyl-leucyl-phenylalanine. The carboxyl-terminal fragment of α_1-AT is also responsible for an increase in synthesis of α_1-AT in human monocytes

and macrophages when these cells are incubated with exogenous neutrophil elastase (73). In each case, the biologic effect is mediated by the interaction of a pentapeptide neodomain within the carboxyl-terminal fragment of α_1-AT and a novel cell-surface receptor, the serpin–enzyme complex (SEC) receptor (74,75,76).

Table 37.3. Deficiency Variants of α_1-Antitrypsin

			Clinical disease		
Variant	**Defect**	**Site**	**Liver**	**Lung**	**Cellular defect**
Z	Single base substitution M1 (Ala213)	Glu342–Lys	+	+	IC accumulation
S	Single base substitution	Glu264–Val	-	-	IC accumulation
$M_{Heerlen}$	Single base substitution	Pro369–Leu	-	+	IC accumulation
$M_{Procida}$	Single base substitution	Leu41–Pro	-	+	IC accumulation
M_{Malton}	Single base	Phe52	?	+	IC

	deletion				accumulation
M_{Duarte}	Unknown	Unknown	?	+	Unknown
S_{Iiyama}	Single base substitution	Ser53–Phe	-	+	?IC degradation
P_{Duarte}	Two base substitutions	Arg101–His Asp256–Val	?	+	Unknown
P_{Lowell}	Single base substitution	Asp256–Val	-	+	?IC degradation
$W_{Bethesda}$	Single base substitution	Ala 336–Thre	-	+	?Accelerated catabolism
$Z_{Wrexham}$	Single base substitution	Ser19–Leu	?	?	Unknown
F	Single base substitution	Arg223–Cys	-	-	Unknown
T	Single base substitution	Glu264–Val	-	-	Unknown
I	Single base substitution	Arg39–Cys	-	-	IC degradation
$M_{Palermo}$	Single base deletion	Phe51	-	-	Unknown
$M_{Nichinan}$	Single base deletion and single base substitution	Phe52 Gly148–Arg	-	-	Unknown
$Z_{Ausburg}$	Single base substitution	Glu342–Lys	-	-	Unknown

IC, intracellular; ?, not proven.

Results of a provocative series of experiments suggested that α_1-AT inhibits human

immunodeficiency virus 1 (77). Separate mechanisms for inhibition of infectivity and production of intact virus were implicated, but the results have not been independently corroborated.

There have been several reports that α_1-AT alters immune function through effects on lymphocytes (2,78). However, there are inherent conflicts in some of the reports, and data have not been duplicated. There is no evidence that the immune response is systemically altered in persons with α_1-AT deficiency.

Biosynthesis of α_1-Antitrypsin

The predominant site of synthesis of plasma α_1-AT is the liver. This is most clearly shown by conversion of plasma α_1-AT to the donor phenotype after orthotopic liver transplantation (79). α_1-AT is synthesized in human hepatocellular carcinoma cells as a 52-kDa precursor; undergoes post-translational, dolichol phosphate–linked glycosylation at three asparagine residues (80); and undergoes tyrosine sulfation (81). It is secreted as a 55-kDa native single-chain glycoprotein with a half-time for secretion of 35 to 40 minutes.

Tissue-specific expression of α_1-AT in human hepatocellular carcinoma cells is directed by structural elements within a 750-nucleotide region upstream of the hepatocyte transcriptional start site in exon I_C. Within these regions are structural elements that are recognized by nuclear transcription factors, including hepatocyte nuclear factor-1α (HNF-1α), HNF-1β, CCAAT/enhancer-binding protein (C/EBP), HNF-4, and HNF-3 (82). HNF-1α and HNF-4 appear to be particularly important for the expression of the human α_1-AT gene in liver cells and intestinal epithelial cells (83). Two distinct regions within the proximal element bind these two transcription factors. Substitution of five nucleotides at positions 77 to 72 disrupts binding of HNF-1α and dramatically reduces expression of the human α_1-AT gene in the liver of transgenic mice (84). Substitution of four nucleotides at positions 118 to 115 disrupts the binding of HNF-4 but does not alter expression of the human α_1-AT gene in the liver of adult transgenic mice. The latter mutation does reduce expression of human α_1-AT in the liver during embryonic development.

Plasma concentrations of α_1-AT increase threefold to fivefold during the host response to inflammation or tissue injury (85). The source of this additional α_1-AT has always been considered the liver; therefore, α_1-AT is known as a *positive hepatic acute-phase reactant*. Synthesis of α_1-AT in human hepatocellular carcinoma cells (e.g., HepG2, Hep3B) is upregulated by interleukin-6 (IL-6) but not by IL-1 or tumor necrosis factor (86). Plasma concentrations of α_1-AT also increase during oral contraceptive therapy and pregnancy (87). α_1-AT is also synthesized and secreted in primary cultures of human blood monocytes and bronchoalveolar and breast milk macrophages (88). Expression of α_1-AT in monocytes and macrophages is influenced by products generated during inflammation, such as bacterial lipopolysaccharide (89) and IL-6 (86).

A series of studies has elucidated a feed-forward regulatory loop that probably represents the dominant mechanism for regulating the synthesis of α_1-AT in cells and tissues. In this regulatory loop, elastase–α_1-AT complexes mediate an increase in the synthesis of α_1-AT through a specific cell-surface receptor (74). The effect on α_1-AT synthesis can be elicited by synthetic peptides corresponding to a domain in the carboxyl-terminal fragment of the α_1-AT molecule that is exposed only after the structural rearrangement that accompanies complex formation or proteolytic modification. These synthetic peptides bind specifically and saturably to a single class of receptors on the cell surface of monocytes and human hepatocellular carcinoma HepG2 cells (dissociation constant [K_d], approximately 40 nmol/L; 4.5×10^5 plasma membrane receptors per cell). This class of receptor molecules is now

called *SEC receptors* because the receptors recognize the highly conserved domains of other SECs, such as antithrombin-III–thrombin, α_1-antichymotrypsin–cathepsin G, and, to a lesser extent, C1 inhibitor–C1s and tissue plasminogen activator–plasminogen activator inhibitor I complexes, as well as that of α_1-AT–elastase complexes (74,90). Substance P, several other tachykinins, bombesin, and the amyloid-β peptide bind to the SEC receptor through a similar pentapeptide sequence (91). Results of our most recent studies indicate that the SEC receptor can mediate endocytosis of soluble amyloid-β peptide, but it does not recognize the aggregated form of amyloid-β peptide, which is toxic to neurons and other cell types (92). Therefore, the SEC receptor may play a role in preventing amyloid-β peptide from accumulating in the amyloid deposits associated with Alzheimer's disease.

α_1-AT mRNA has been isolated from several tissues in transgenic mice (93), but in many cases it has not been possible to determine whether this mRNA is in ubiquitous tissue macrophages or other cell types. α_1-AT is synthesized in enterocytes and Paneth cells, as indicated by the results of studies with intestinal epithelial cell lines, ribonuclease protection assays of human intestinal ribonucleic acid (RNA), and in situ hybridization analysis in cryostat sections of human

intestinal mucosa (94). Expression of α_1-AT in enterocytes increases markedly during differentiation from crypt to villus, in response to IL-6, and during inflammation in vivo. α_1-AT is also synthesized by pulmonary epithelial cells (95,96). Synthesis of α_1-AT in pulmonary epithelial cells is less responsive to regulation by IL-6 than by a related cytokine, oncostatin M (96). Moreover, there is evidence that HNF-1β, and not HNF-1α, HNF-4, or HNF-3, plays a predominant cell-specific role in transcription of the α_1-AT gene in pulmonary epithelial cells (97).

Clearance and Distribution

The half-life of α_1-AT in plasma is approximately 5 days (98). It is estimated that the daily production rate of α_1-AT is 34 mg/kg body weight, 33% of the intravascular pool of α_1-AT being degraded daily. There is a slight increase in the rate of clearance of radiolabeled PIZ α_1-AT compared with that of PIM α_1-AT when infused into PIMM individuals, but this difference does not account for the decrease in serum levels of α_1-AT in persons with the deficiency (99). The low-density lipoprotein receptor–related protein (LRP) family probably plays a major role in the clearance and catabolism of α_1-AT when it is in complex with neutrophil elastase (100,101). The SEC receptor may be involved in the clearance and catabolism of both complex and modified forms of α_1-AT (90,102), but this has not yet been tested in vivo. The mechanism of clearance of native α_1-AT is not yet known (103).

α_1-AT diffuses into most tissues and is found in most body fluids (55). Its concentration in lavage fluid from the lower respiratory tract is approximately equivalent to that in serum. α_1-AT is also found in feces, and increased fecal concentrations of α_1-AT correlate with the presence of inflammatory lesions of the bowel (104). In each case, it is assumed that α_1-AT is derived from serum. However, local sites of synthesis, such as macrophages and epithelial cells, may make important contributions to the α_1-AT pool in these tissues and body fluids.

Pathogenesis of Liver Injury in PIZZ Individuals

There are several theories for the pathogenesis of liver injury in α_1-AT deficiency. According to the *immune theory*, liver damage results from an abnormal immune response to liver antigens (105). This theory is based on the observation that peripheral blood lymphocytes from PIZZ infants are cytotoxic for isolated hepatocytes; however, this is probably a nonspecific effect of liver injury, in that peripheral blood lymphocytes from PIMM infants with a similar degree of liver injury

due to idiopathic neonatal hepatitis syndrome are also cytotoxic for isolated hepatocytes. More recent studies have indicated an increase in the human leukocyte antigen (HLA)-DR3-DW25 haplotype in α_1-AT–deficient persons with liver disease (106). However, there is no difference between the expression of major histocompatibility complex (MHC) class II antigen in the livers of these persons and its expression in healthy controls (107). Moreover, an increase in the prevalence of a particular HLA-DR haplotype in the affected population does not by itself imply altered immune function. Because of the linkage disequilibrium displayed by genes within the MHC, it is possible that increased susceptibility is caused by the products of unrelated but linked genes. For example, the MHC contains genes for several heat-shock/stress proteins (108), which play an important role in the biogenesis and transport of other proteins through the secretory pathway.

The *accumulation theory*, in which liver damage is thought to be caused by accumulation of mutant α_1-AT molecules in the ER of liver cells, is the most widely accepted. Experimental results with transgenic mice are most consistent with this theory and completely exclude the possibility that liver damage is caused by "proteolytic attack" as a consequence of diminished serum α_1-AT concentrations. Transgenic mice carrying the mutant Z allele of the human α_1-AT gene develop periodic acid–Schiff-positive, diastase-resistant intrahepatic globules and liver injury early in life (109,110). Because there are normal levels of α_1-AT and presumably other antielastases in these animals, as directed by endogenous murine genes, the liver injury cannot be attributed to proteolytic attack. Therefore, liver damage in this deficiency is thought to involve a gain-of-toxic function mechanism.

It has been difficult to reconcile the accumulation theory with the observations of Sveger and Eriksson (8,9), who showed that only a subset of PIZZ α_1-AT–deficient persons sustain marked liver damage. The author and his colleagues have predicted that a subset of the PIZZ population is more susceptible to liver injury through the presence of one or more additional inherited traits or environmental factors that exaggerate the intracellular accumulation of the mutant Z α_1-AT protein or exaggerate the cellular pathophysiologic consequences of mutant α_1-AT accumulation (111). A direct examination of this prediction follows.

Mechanism for Deficiency of α_1-Antitrypsin in PIZZ Individuals

The mutant α_1-ATZ molecule is characterized by a single nucleotide substitution that results in a

single–amino acid substitution, Lys for Glu342 (112,113,114). There is a selective decrease in the secretion of α_1-AT, the abnormal protein accumulating in the ER (115,116,117,118). The defect is not specific to liver cells because it also affects extrahepatic sites of α_1-AT synthesis, such as macrophages (115) and transfected cell lines (117,118,119,120). Site-directed mutagenesis studies have shown that this single–amino acid substitution is sufficient to produce the cellular defect (118,119,120). Once translocated into the lumen of the ER, the mutant α_1-AT protein is unable to traverse the remainder of the secretory pathway because it is abnormally folded.

Several studies have provided evidence that the substitution of Glu342 by Lys in the α_1-ATZ variant decreases the stability of the molecule in its monomeric form and increases the likelihood that it will form polymers by means of a so-called loop-sheet insertion mechanism (99). In this mechanism, the reactive center loop of one α_1-AT molecule inserts into a gap in the β-pleated A sheet of another α_1-AT molecule (Fig. 37.2). Lomas et al. (121) were the first to notice that the site of the amino acid substitution in the PIZ α_1-AT variant was at the base of the reactive center loop,

adjacent to the gap in the A sheet. These investigators predicted that a change in the charge at this residue, as occurs with the substitution of Lys for Glu, would prevent insertion of the reactive-site loop into the gap in the A sheet during interaction with enzyme; therefore, the mutant α_1-ATZ would be susceptible to the insertion of the reactive center loop of adjacent molecules into the gap in its A sheet. This would cause the mutant α_1-ATZ to be more susceptible to polymerization than the wild-type α_1-AT. The results of these experiments showed that α_1-ATZ undergoes this form of polymerization spontaneously to a certain extent, and to a greater extent during relatively minor perturbations, such as an increase in temperature. Presumably, an increase in body temperature during systemic inflammation would exacerbate this tendency in vivo. Polymers have also been detected by means of electron microscopic examination of the ER of hepatocytes in a liver biopsy specimen from a PIZZ individual (121). Similar polymers have been found in the plasma of patients with the PIS_{Iiyama} α_1-AT variant and the PIM_{Malton} α_1-AT variant (122,123). The mutations in α_1-AT PIS_{Iiyama} (Ser53 to Phe) (53) and α_1-ATPIM_{Malton} (Phe52 deletion) (17) affect residues that provide a ridge for the sliding movement opening the A sheet. Therefore, these mutations would be expected to interfere with the insertion of the reactive center loop into the gap in the A sheet and therefore leave the gap in the A sheet available for spontaneous loop-sheet polymerization. It is interesting that the hepatocytic α_1-AT globules have been found in a few patients with these two variants. Recent observations suggest that the α_1-ATS variant also undergoes loop-sheet polymerization (124) and that this may account for its retention in the ER, although to a milder extent than that for α_1-ATZ (49).

Loop-sheet insertion also appears to be responsible for polymerization of other serpins in clinical deficiency states, including antithrombin deficiency (125) and C1 inhibitor deficiency (126). A striking example of this phenomenon is the familial dementia associated with Collins bodies. Studies by Davis et al. (127) have shown that these neuronal inclusion bodies contain a polymerized mutant neuroserpin.

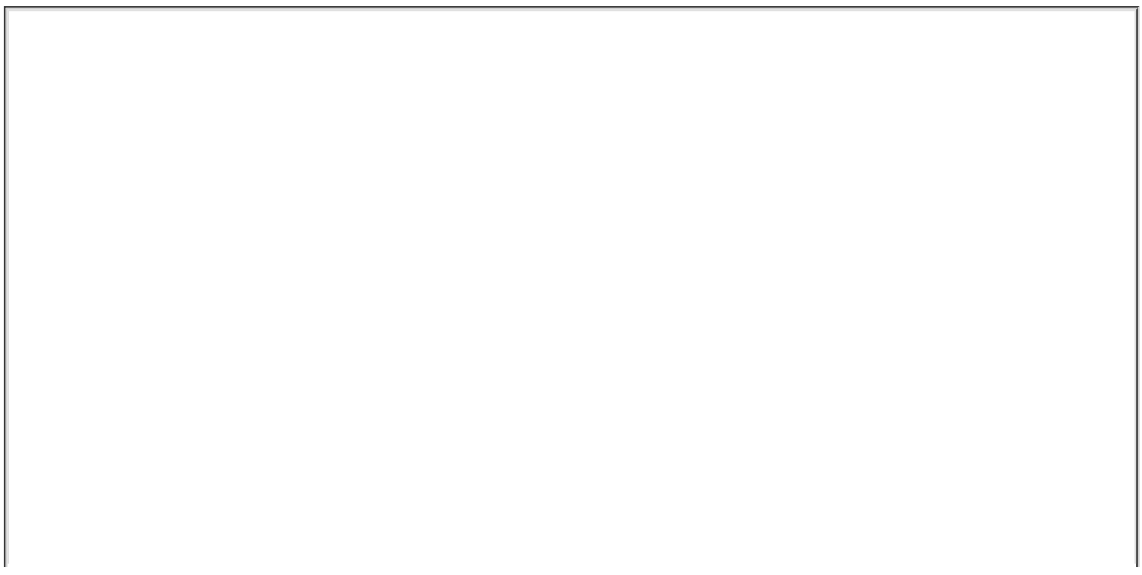
The precise mechanism by which the loop-sheet insertion develops is not yet completely understood and may be more complicated than previously predicted (123). Further studies to characterize the mechanism more precisely will, undoubtedly, be forthcoming.

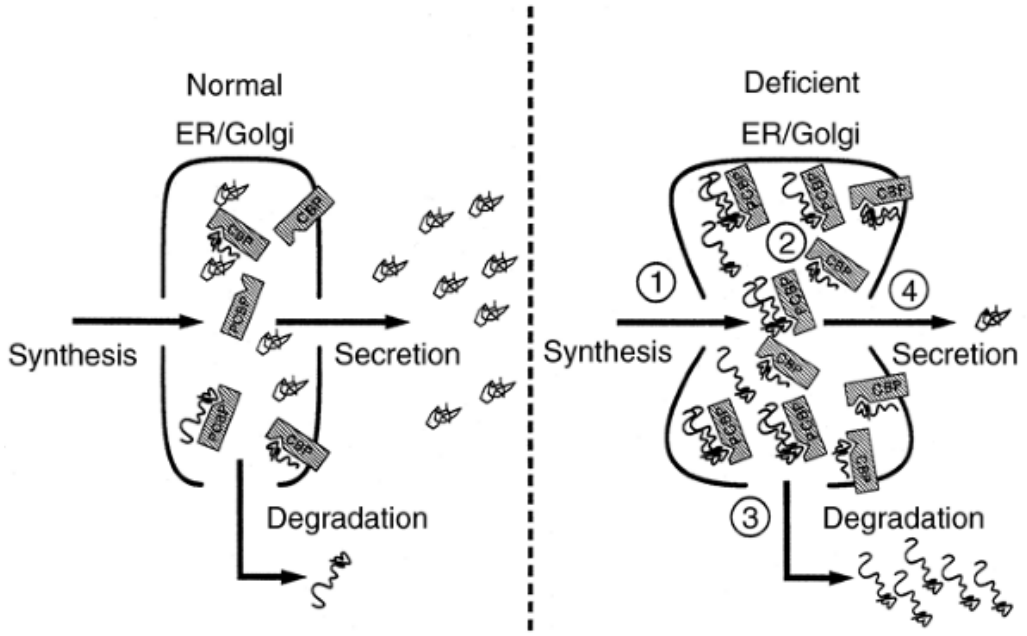
Yu et al. (128) compared the folding kinetics of α_1-ATZ in transverse urea gradient gels. The results of the study showed for the first time that α_1-ATZ folds at an extremely slow rate, unlike the wild-type α_1-AT, which folds in minutes. This folding defect leads to the accumulation of an intermediate that has a high tendency to polymerize, presumably by the loop-sheet insertion mechanism.

Several subsequent studies have suggested that polymerization is the cause of retention of α_1-ATZ in the ER of liver cells. The most powerful of these are studies showing partial correction of the secretory defect by insertion of a second mutation into the α_1-ATZ protein that suppresses loop-sheet polymerization (129,130,131). However, these studies do not exclude the possibility that there is an abnormality in folding that is distinct from the tendency to polymerize and is also partially corrected by the second, experimentally introduced mutation. More recent studies cast some doubt on the concept that polymerization is the cause of ER retention. First, naturally occurring variants of α_1-AT, in which the carboxyl-terminal tail is truncated, including a double mutant with the substitution that characterizes the Z allele together with the substitution that results in carboxyl-terminal truncation, are retained in the ER of liver cells although they do not form polymers (45). Second, only a minor proportion (approximately 18%) of the intracellular pool of α_1-ATZ at steady state in model cell lines is in the form of polymers (45,132). Moreover, because the remainder of α_1-ATZ in the ER in vivo is in the form of heterogeneous

soluble complexes with multiple ER chaperones (132), the principles by which purified α_1-ATZ polymerizes in vitro are probably not applicable to what happens in live cells in vivo. Taken together, the extant data suggest that polymerization is not the cause by which α_1-ATZ is retained in the ER of liver cells but rather is the result of its retention. Nonetheless, the polymerogenic properties of the Z mutant are still likely to be critical determinants in the pathobiology of liver disease.

To understand how polymerization of α_1-AT or alteration in the folding of monomeric α_1-AT might result in retention within the ER, we must consider what is known about the biology of protein secretion. Most newly synthesized secretory proteins are translocated into the lumen of the ER together with membrane proteins. Before being transported to their final destination, these nascent secretory and membrane polypeptide chains undergo a series of post-translational modifications, including glycosylation, formation of disulfide bonds, oligomerization, and folding. It is still not clear, however, whether the mechanism by which the secretory proteins are transported out of the ER involves one of three possible alternatives: (a) The protein encodes a transport signal by which it is recognized for selective removal from the ER and is concentrated as cargo in a departing vesicle, (b) the protein encodes a signal for retention in the ER and restricts it from entering a departing vesicle, or (c) the protein lacks both transport and retention signals and enters, by means of bulk flow, the budding vesicles at its prevailing concentration in the ER (133). Recent studies have provided evidence for the involvement of each of these mechanisms (134). Several cargo receptors, which direct transport out of the ER, have been identified, but so far we know of only a select group of secretory protein ligands governed by this mechanism. Retention within the ER is thought to constitute the quality control apparatus of the cell by which incompletely assembled, improperly folded, and mutant proteins are recognized and ultimately degraded to prevent them from causing damage to the cell. Several families of resident ER proteins, termed *molecular chaperones*, appear to be involved in this quality control apparatus. One family has been referred to as the *polypeptide chain binding protein family* and includes several heat-shock and heat-stress proteins, GRP78/BiP and GRP94, protein disulfide isomerase, and Erp72 (135). Several calcium-binding phosphoproteins of the ER, most notably calnexin and calreticulin, also have molecular chaperone activity within the ER. Calnexin is an approximately 88-kDa transmembrane ER resident phosphoprotein that was originally discovered in association with MHC class I molecules (136). It facilitates the assembly of the MHC class I with antigenic peptide. It has also been shown to interact transiently with folding and assembly intermediates of a wide array of soluble proteins (137).





▪ **Figure 37.4** Conceptual model for liver injury in α_1-antitrypsin (α_1-AT) deficiency. Polypeptide chain–binding proteins (*PCBP*) and membrane-associated calcium-binding proteins (*CBP*) are shown as *hatched bars*. The α_1-AT molecules that are unfolded or misfolded are shown as *long wavy lines*, and those that are maturely folded are shown as *condensed masses*. In the normal state, α_1-AT enters the secretory pathway (endoplasmic reticulum [*ER*]) unfolded. Interaction with PCBP or CBP facilitates folding into a mature state and allows dissociation from the PCBP or CBP in a form competent for secretion. A relatively minor proportion of the α_1-AT molecules that do not fold into the mature state are directed into a pathway for degradation in, or through, the ER. In a person with α_1-AT deficiency, only a minor proportion of the mutant α_1-AT molecules achieve the mature, folded state that allows secretion. There is a net accumulation of α_1-AT molecules in the ER and an increase in α_1-AT molecules directed into the pathway for degradation. Further intracellular accumulation of α_1-AT, which is potentially hepatotoxic, could result from increased synthesis of α_1-AT (1), abnormalities in interaction with PCBP or CBP (2), abnormalities in degradative enzymes (3), or abnormalities in bulk flow that allow secretion (4) of 10% to 15% of the newly synthesized mutant α_1-ATZ molecules.

Polymerization of α_1-ATZ or alteration in the folding of monomeric α_1-ATZ could result in ER retention because it prevents an essential transport signal from being recognized or because it newly exposes a signal for retention (Fig. 37.4). Net retention of mutant α_1-ATZ in the ER also depends, to a certain extent, on its rate of degradation in the ER. A number of studies have provided evidence of degradative systems in the ER and evidence that such systems are involved in the degradation of misfolded or incompletely assembled proteins (137). Any defect in the interaction between α_1-ATZ and ER molecular chaperones or in an ER degradation pathway could predispose a person

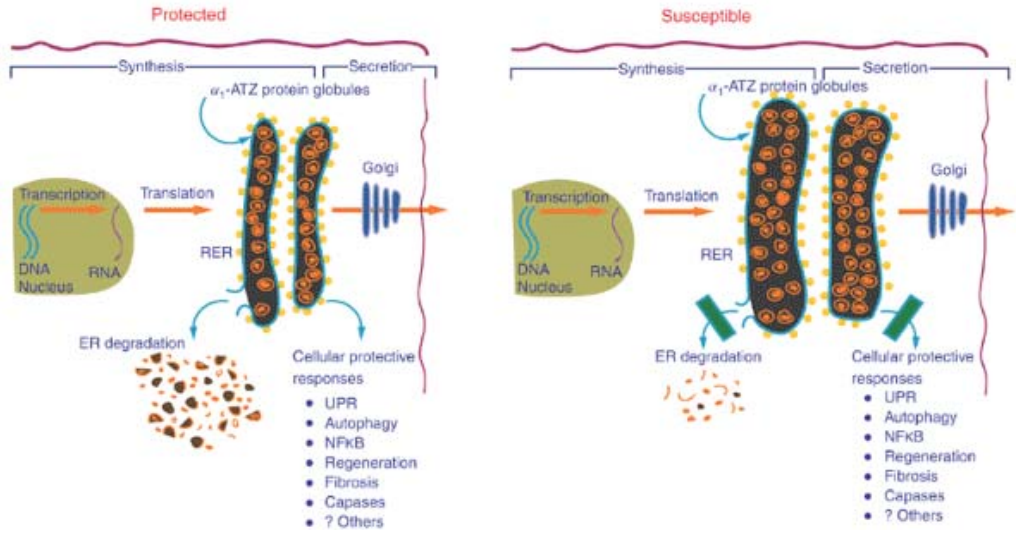
with α_1-AT deficiency to greater accumulation of the presumably hepatotoxic α_1-ATZ molecule in the ER (Fig. 37.5). Such a defect, whether determined by a genetic trait or an environmental factor, would be silent in the general population, which is not

exposed to a chronic burden of mutant, misfolded secretory protein.

With these considerations in mind, the author and his colleagues predicted a number of years ago that any unlinked genetic trait or environmental factor that impairs ER degradation would predispose a subgroup of α_1-AT deficient, PIZZ individuals to liver injury (111). To address this prediction experimentally, we transduced skin fibroblasts from PIZZ individuals, with or without liver disease, with amphotropic recombinant retroviral particles designed for constitutive expression of the mutant α_1-ATZ gene. Human skin fibroblasts do not express the endogenous α_1-AT gene but are presumed to express other genes involved in the postsynthetic processing of secretory proteins. The results show that expression of the human α_1-AT gene was conferred on each fibroblast cell line. Compared with the effect when the same cell line was transduced with the wild-type α_1-AT gene, there was selective intracellular retention of the mutant α_1-ATZ protein in each case. However, there was a marked delay in the degradation of the mutant α_1-ATZ protein after it accumulated in the fibroblasts from PIZZ individuals with liver disease (susceptible hosts) compared with the effect in PIZZ individuals without liver disease (protected hosts) (Fig. 37.5). These data provide evidence that other factors that affect the fate of the abnormal α_1-ATZ molecule, such as a lag in ER degradation, at least in part determine susceptibility to liver disease.

Because the pathway for degradation of α_1-ATZ that is retained in the ER is an obvious candidate for genetic variations associated with protection from, or susceptibility to, liver injury by the gain-of-toxic function mechanism, the author, his colleagues, and others have sought to characterize the pathway. The results indicate that it is much more complex than initially envisioned, with multiple mechanisms depending on a number of characteristics such as the specific substrate and the concentration of that substrate in the ER. Studies in multiple laboratories and in multiple systems have indicated that the proteosome is involved (137,138,139,140,141,142). The proteasome is a large cytoplasmic multiprotein complex that degrades many cellular proteins, particularly denatured proteins. Degradation by the proteasome depends on adenosine triphosphate; involves trypsin-like, chymotrypsin-like, and peptide glutamyl peptide hydrolyzing activity; and, for the most part, targets proteins that have been conjugated by ubiquitin. Although it is relatively easy to conceptualize how a transmembrane protein such as CFTRΔF508 might be accessible on the cytoplasmic aspect of the ER membrane for ubiquitination and degradation by the proteasome, it is more difficult to conceptualize how this might occur for a luminal polypeptide, such as α_1-ATZ. To address this issue, we used a cell-free system and found that α_1-ATZ must interact with the transmembrane molecular chaperone calnexin and induce ubiquitination of the cytoplasmic tail of calnexin to

undergo degradation by the proteasome (138). Results of further studies have indicated that there are at least two other mechanisms for ER degradation of α_1-ATZ, including a ubiquitin-independent proteasomal mechanism and one or more nonproteasomal mechanisms. The ubiquitin-dependent mechanism, in which the α_1-ATZ–calnexin complex is targeted, requires recruitment of the ubiquitin conjugating enzyme E2-F1 from the cytoplasm onto the ER membrane and polybiquitination of the cytoplasmic tail of calnexin (138). Studies in yeast (137) have also shown that calnexin and the proteasome are involved in ER degradation of α_1-ATZ.



▪ **Figure 37.5** Difference in endoplasmic reticulum (*ER*) degradation of α_1-ATZ protein in protected and susceptible hosts. The blocks in ER degradation and cellular response pathways in susceptible hosts are represented by *small dark bars*. RER, rough endoplasmic reticulum; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; UPR, unfolded protein response; ?, not proven.

We do not yet know exactly how the entire α_1-ATZ–calnexin complex, including the luminal domain of calnexin associated with α_1-ATZ, is degraded. The proteasome may initiate a process that is completed by other enzymes within the ER membrane or within the ER lumen. Several other mechanisms by which the ubiquitin system and the proteasome gain access to membrane-bound and luminal substrates of the ER degradation pathway have been discussed in the literature. Although the retrograde translocation mechanism in which substrates are transported from the ER lumen to the cytoplasm had been demonstrated for some luminal substrates of the proteosome, limited evidence exists for retrograde translocation of α_1-ATZ. Werner et al. (137) detected α_1-ATZ in the cytosolic fraction of yeast when the proteosome was inhibited, but it was only a small fraction of the total α_1-ATZ in the ER and there has been no other evidence for retrotranslocation. This may mean that other mechanisms provide for transporting α_1-ATZ from the ER to the cytoplasm such as proteosome-mediated extraction through the ER membrane, as has been demonstrated for model ER degradation substrates (143). This "membrane extraction" mechanism, or "dislocation" mechanism, may be particularly relevant to degradation of α_1-ATZ because ER degradation of this substrate appears to involve polyubiquitination on the cytoplasmic tail of the transmembrane ER chaperone calnexin only when it has bound α_1-ATZ at the luminal surface of the ER membrane. It is also possible that the proteasome gains access to α_1-ATZ or the α_1-ATZ–polyubiquitinated calnexin during the formation of autophagic vacuoles. Our studies have shown that retention of α_1-ATZ in the ER is associated with the induction of an autophagic response (144), which is thought to be a general mechanism by which intracellular organelles, or parts of organelles, are degraded. It is a highly evolutionarily conserved process that occurs in many cell types, especially during stress states, such as nutrient deprivation, and during the cellular remodeling that accompanies morphogenesis, differentiation, and senescence. Results of several studies have suggested that autophagic vacuoles are derived in part from subdomains of ER (145). Autophagosomes initially form as invaginations from

ribosome-free areas of the ER membrane. Together with constituents of the ER, autophagosomes engulf cytosolic constituents, including components of the ubiquitin system and the proteasome (146,147). Therefore, it is possible that degradation of α_1-ATZ is mediated by proteasomal machinery engulfed during the formation of the autophagosome. In our studies an intense autophagic response was found in cell culture model systems with ER retention of mutant α_1-ATZ and in liver biopsy specimens from patients with α_1-AT deficiency (144). Moreover, α_1-ATZ and calnexin were colocalized within autophagosomes, as well as within the ER. Finally, degradation of α_1-ATZ in the cell culture model system is partially abrogated by inhibitors of autophagy, including wortmannin, 3-methyladenine, and LY294002, and recently, degradation of α_1-ATZ was abrogated in a cell line genetically engineered to be deficient in autophagy (141). However, it is also possible that α_1-ATZ molecules taken up into autophagosomes are degraded by a nonproteasomal mechanism when the autophagosomes merge or fuse with the lysosomal pathway and that the autophagic and proteasomal pathways constitute completely independent mechanisms for degradation of α_1-ATZ.

Several possible nonproteasomal mechanisms could contribute to ER degradation of mutant α_1-ATZ. In addition to the autophagic pathway (111), there is a mechanism, described by Cabral et al. that is sensitive to tyrosine phosphatase inhibitors (148). These authors found that degradation of α_1-ATZ in a transfected mouse hepatocellular carcinoma cell line was not sensitive to proteasomal inhibitors but was sensitive to tyrosine phosphatase inhibitors, suggesting that this nonproteasomal mechanism was the predominant pathway for ER degradation of α_1-ATZ in liver cells. Studies in the author's laboratory have shown that degradation of α_1-ATZ in the same hepatocellular carcinoma cell line, as well as in other lines, is sensitive to proteasomal inhibitors (140). These findings indicate that the mechanism observed by Cabral et al. is probably cell line specific rather than cell type specific for hepatocytes.

Together the results of the foregoing studies indicate that degradation of α_1-ATZ is a complex process that may involve more than one pathway and at least several sequential steps in each pathway. Theoretically, each of these pathways or its individual steps may be affected in an α_1-AT–deficient patient who is "susceptible" to liver disease; that is, there may be heterogeneity among susceptible hosts in the mechanism by which ER degradation is delayed.

Mechanism of Liver Injury

There is still relatively little information about the mechanism by which ER retention of α_1-ATZ leads

to liver cell injury. In transgenic mice that express the human α_1-ATZ gene, there are focal areas of liver cell necrosis, microabcesses with an accumulation of neutrophils, regenerative activity in the form of multicellular liver plates, and focal nodule formation during the neonatal period (149). Nodular clusters of altered hepatocytes that lack α_1-AT immunoreactivity are also found during the neonatal period. With aging, there is a decrease in the number of hepatocytes containing α_1-ATZ globules; there is also an increase in the number of nodular aggregates of α_1-AT–negative hepatocytes and development of perisinusoidal fibrosis (149). Within 6 weeks, there are dysplastic changes in these aggregates. Adenoma occurs within 1 year, and invasive hepatocellular carcinoma occurs between 1 and 2 years of age (150). The histopathology of α_1-ATZ transgenic mice is remarkably similar to that of hepatitis B virus surface antigen in transgenic mice and is particularly interesting because hepatitis B virus is retained in the ER or the ER–Golgi intermediate compartment of hepatocytes, often called *ground-glass hepatocytes* (151). It is still

unclear why the liver injury in the transgenic mouse model of α_1-AT deficiency is somewhat milder and less fibrogenic than that in children with α_1-AT deficiency–associated liver disease. It is possible that strain-specific factors condition the response to injury, just as there are apparently host-specific factors that affect the response to injury in α_1-AT–deficient infants (111).

In one of the recent studies by the author and colleagues we found evidence for hepatic mitochondrial injury in α_1-AT deficiency (152), raising the possibility that liver damage is mediated by oxidative mechanisms. Mitochondrial damage was evident in both cell line and transgenic mouse models of α_1-AT deficiency including mitochondrial depolarization and activation of caspase-3. Treatment of the PIZ mouse model with cyclosporin A, an inhibitor of mitochondrial depolarization, resulted in less histologic damage and complete reversal of mortality associated with the experimental stress of starvation (152).

In another recent study, Rudnick et al. examined the proliferation of hepatocytes in the PIZ mouse model (153), and the results have led to a new theory for the mechanism by which α_1-AT deficiency is predisposed to hepatocellular carcinoma. This study showed that there was increased hepatocellular proliferation in the liver at baseline. The increase was 5- to 10-fold above that in controls and highly significant statistically; it did represent a relatively low number of BrdU-positive hepatocytes (2% to 3% detected over 72 hours of continuous labeling). These data indicate that liver injury in the mouse model is relatively mild and appropriately corresponds to the smoldering and slowly progressing liver disease seen in most α_1-AT–deficient patients. Four other important observations were made. First, the increase in proliferation was proportional to the number of hepatocytes with globules containing the retained α_1-ATZ. This suggested that the globule-containing hepatocytes were producing a regenerative signal or signals. Second, the proliferating hepatocytes were almost entirely the ones devoid of globules. This suggested that retention of α_1-ATZ inhibits cell proliferation and that cells with lesser accumulation of α_1-ATZ have a selective proliferative advantage in the damaged liver. Third, both globule-containing and globule-devoid hepatocytes proliferated when the PIZ mice were subjected to partial hepatectomy. This suggested that the block in proliferation of globule-containing hepatocytes is relative; that is, that they would proliferate if the stimulus were as powerful as the one generated after partial hepatectomy. Fourth, globule-containing hepatocytes were not disproportionately affected by signs of cell death. This suggested that the globule-containing hepatocytes were "sick but not dead."

These observations constitute the basis for a new paradigm for the pathogenesis of hepatic cancer in α_1-AT deficiency and, perhaps, other chronic liver diseases. Globule-devoid hepatocytes, which are probably progenitors or at least young cells with lesser time to accumulate the mutant protein, have a selective proliferative advantage in the liver of the deficient individual. They are chronically stimulated in "trans" by signals that are generated by globule-containing hepatocytes that are "sick but not dead." The cancer-prone state is then engendered by having cells that are unable to die at the appropriate time and cells that are chronically dividing in the inflamed milieu. This paradigm is consistent with what has been found in the liver of the Z#2 mouse model of α_1-AT deficiency (150). Most of the liver (>90%) becomes α_1-AT negative as the mouse ages. This probably represents the selective proliferation of the globule-devoid hepatocytes, the progenitor cells. Adenomas and then carcinomas arise in these regions in greater than 80% of the mice. This paradigm also appears to apply to the predilection for hepatic cancer in several other forms of chronic liver disease (154).

Further understanding of how hepatocytes respond to the accumulation of α_1-ATZ in the ER is likely to provide more clues about the pathogenesis of this liver disease.

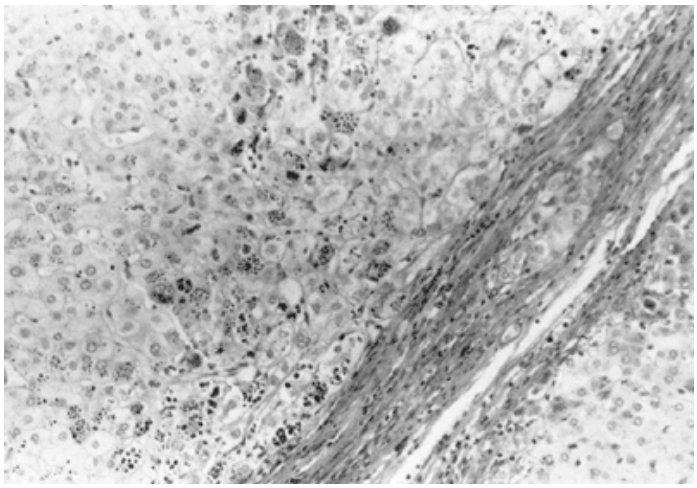
Hidvegi et al. have used cell line and transgenic mouse models with inducible expression of mutant α_1-ATZ, which are ideally suited for elucidating the signal transduction pathways that are activated and for determining how they may protect from, or contribute to, liver damage (155). So far these studies have shown that accumulation of the mutant protein in the ER of the model systems does not activate the unfolded protein response but does activate autophagy, nuclear factor κB (NFκB), ER-caspases and BAP31, an ER protein that appears to be involved in the proapoptotic effects of ER on mitochondria. The latter may be the mechanism by

which mitochondrial damage and mitochondrial caspases are activated in the liver in α_1-AT deficiency. The rest of this signaling profile is also consistent with the conceptual model of the globule-containing hepatocytes as "sick but not dead" (154).

Diagnosis

Diagnosis of α_1-AT deficiency is established by means of serum α_1-AT phenotype determination in isoelectric focusing or by means of agarose electrophoresis at acid pH. Serum concentrations can be used for screening, with follow-up PI typing of any values below normal (85 to 215 mg/dL). A retrospective study of all pediatric patients who had both serum concentrations and PI typing done at one center indicated that the serum concentration determination had a positive predictive value of 94% and a negative predictive value of 100% for homozygous α_1-AT deficiency (156). However, because of the inherent limitations of retrospectively defining a patient population for the analysis, the results of the study are not necessarily applicable to each diagnostic situation that might be encountered. It is wise to get a phenotype together with the serum level in most cases of neonatal hepatitis or unexplained chronic liver disease in older children, adolescents, and adults. Serum concentrations of α_1-AT may be helpful, when used with the phenotype, to differentiate persons homozygous for the Z allele from SZ compound heterozygotes, both of whom may develop liver disease. In some cases, phenotype determinations of parents and other relatives are necessary to ensure the distinction between ZZ and SZ allotypes, a distinction important for genetic counseling. Serum concentrations of α_1-AT are occasionally misleading. For example, serum α_1-AT concentrations may increase during the host response to inflammation, even in homozygous PIZZ individuals and give a falsely reassuring impression.

The distinctive histologic feature of homozygous PIZZ α_1-AT deficiency, periodic acid–Schiff-positive, diastase-resistant globules in the ER of hepatocytes, substantiates the diagnosis (Fig. 37.6). The presence of these inclusions should not be interpreted as confirming the diagnosis of α_1-AT deficiency. Similar structures are occasionally found in PIMM individuals with other liver diseases (157). The inclusions are eosinophilic, round to oval, and 1 to 40 μm in diameter. They are most prominent in periportal hepatocytes but may also be present in Kupffer cells and cells of biliary ductular lineage (158). There may be evidence of variable degrees of hepatocellular necrosis, inflammatory cell infiltration, periportal fibrosis, or cirrhosis. There may also be evidence of bile duct epithelial cell destruction, and occasionally there is a paucity of intrahepatic bile ducts.



▪ **Figure 37.6** Histologic appearance of liver biopsy specimen of a patient with homozygous PIZZ α_1-AT deficiency. Micrograph shows periodic acid–Schiff-positive, diastase-resistant globules in hepatocytes, especially periportal ones, adjacent to a broad band of fibrous tissue (periodic acid–Schiff, diastase, 40× original magnification). (Photomicrograph provided by Dr. C. Coffin, St. Louis, MO.)

Treatment

The most important principle in the treatment of patients with α_1-AT deficiency is avoidance of cigarette smoking, which markedly accelerates the destructive lung disease associated with α_1-AT deficiency, reduces the quality of life, and significantly shortens the longevity of these patients (2).

There is no specific therapy for α_1-AT deficiency–associated liver disease. Therefore, clinical care largely involves supportive management of symptoms caused by liver dysfunction and prevention of complications. Progressive liver dysfunction and failure in children have been managed with orthotopic liver transplantation, with survival rates approaching 90% at 1 year and 80% at 5 years (159). Nevertheless, a number of PIZZ individuals with severe liver disease, even cirrhosis or portal hypertension, may have a relatively low rate of disease progression and lead a relatively normal life for extended periods. With the availability of living–related-donor transplantation techniques, it may be possible to treat these patients expectantly for some time. Children with α_1-AT deficiency, mild liver dysfunction (elevated transaminase levels or hepatomegaly), and without functional impairment may never need liver transplantation.

Several studies have shown that a class of compounds called *chemical chaperones* can reverse the cellular mislocalization or misfolding of mutant plasma membrane, and lysosomal, nuclear, and cytoplasmic proteins, including CFTRΔF508, prion proteins, mutant aquaporin molecules associated with nephrogenic diabetes insipidus, and mutant galactosidase

A associated with Fabry's disease (160,161,162). These compounds include glycerol, trimethylamine oxide, deuterated water, and 4-phenylbutyric acid (PBA). The author and his colleagues have found that glycerol and PBA mediate a marked increase in the secretion of α_1-ATZ in a model cell culture system (163). Oral administration of PBA was also well tolerated by PIZ mice (transgenic for the human α_1-ATZ gene) and

consistently mediated an increase in blood levels of human α_1-AT, reaching 20% to 50% of the levels present in PIM mice and healthy humans. PBA did not affect the synthesis or intracellular degradation of α_1-ATZ. The α_1-ATZ secreted in the presence of PBA was functionally active, in that it could form an inhibitory complex with neutrophil elastase. Because PBA has been used safely for years as an ammonia scavenger to treat children with urea cycle disorders and because results of clinical studies have suggested that only partial correction of the deficiency state is needed for the prevention of both liver and lung injury in α_1-AT deficiency (164), PBA is an excellent candidate for the chemoprophylaxis of target-organ injury in α_1-AT deficiency.

It also appears now that several iminosugar compounds may be useful for chemoprophylaxis of liver and lung disease in α_1-AT deficiency. These compounds are designed to interfere with oligosaccharide side chain trimming of glycoproteins and are being examined as potential therapeutic agents for viral hepatitis and other types of infection (165,166). The author and colleagues examined several of these compounds to determine the effect of inhibition of glucose or mannose trimming of carbohydrate side chains on the fate of α_1-ATZ in the ER. They found, to their surprise, that one glucosidase inhibitor, castanospermine (CST), and two α-mannosidase I inhibitors, kifunensine (KIF) and deoxymannojirimicin (DMJ), mediated increased secretion of α_1-ATZ (167). The α_1-ATZ secreted in the presence of these drugs has partial functional activity. KIF and DMJ are less attractive candidates for chemoprophylactic trials because they delay degradation of α_1-ATZ and increase its secretion. These drugs, therefore, have the potential to exacerbate susceptibility to liver disease. However, CST has no effect on the degradation of α_1-ATZ and, therefore, may be targeted for development as a chemoprophylactic agent. The mechanism of action of CST on α_1-ATZ secretion is not yet known. An interesting hypothesis for the mechanism of action of KIF and DMJ is that mutant α_1-ATZ interacts with ERGIC-53 for transport from ER to Golgi apparatus when mannose trimming is inhibited.

Patients with α_1-AT deficiency and emphysema have undergone replacement therapy with α_1-AT purified from recombinant plasma and administered intravenously or by means of intratracheal aerosol (40). This therapy is associated with improvement in α_1-AT serum concentrations and in α_1-AT and neutrophil elastase inhibitory capacity in bronchoalveolar lavage fluid without significant side effects. Although results of initial studies have suggested that there is a slower decline in forced expiratory volume in patients undergoing replacement therapy, this occurred only in a subgroup of patients, and the study was not randomized (168). This therapy is designed for persons with established and progressive emphysema. Protein replacement therapy is not being considered for patients with liver disease because there is no information to support the notion that deficient serum levels of α_1-AT are mechanistically related to liver injury.

A number of patients with severe emphysema from α_1-AT deficiency have undergone lung transplantation in the last 10 years. The latest data from the St. Louis International Lung Transplantation Registry show that 91 patients with emphysema and α_1-AT deficiency underwent single or bilateral lung transplantation by 1993. The actuarial survival rate among patients in this category who underwent transplantation between 1987 and 1994 was approximately 50% for 5 years. Lung function and exercise tolerance were significantly improved (169).

Replacement of α_1-AT by means of somatic gene therapy has been discussed in the literature (40). This strategy is potentially less expensive than replacement therapy with purified protein and can alleviate the need for intravenous or inhalation therapy. Again, this form of therapy will be useful only in ameliorating emphysema because liver disease associated with α_1-AT deficiency is not caused by deficient

levels of α_1-AT in the serum or tissue. Of course, it would be helpful to know whether replacement therapy with purified α_1-AT, as it is currently applied, is effective in ameliorating emphysema in this deficiency before embarking on clinical trials involving gene therapy. There are still major issues that must be addressed before gene therapy becomes a realistic alternative (170). Several novel types of gene therapy, such as repair of mRNA by means of trans-splicing ribozymes (171,172) and chimeric RNA/DNA oligonucleotides (173,174,175), triplex-forming oligonucleotides (176), small fragment homologous replacement (177), or RNA silencing (178,179), are theoretically attractive alternative strategies for the management of liver disease in α_1-AT deficiency because they would prevent the synthesis of mutant α_1-ATZ protein and ER retention.

Studies have shown that transplanted hepatocytes can repopulate the diseased liver in several mouse models, including a mouse model of a childhood metabolic liver disease called *hereditary tyrosinemia* (180,181). Stem cells from the bone marrow or pancreas can be used instead of adult hepatocytes (182,183). Replication of the transplanted cells occurs only when there is injury or regeneration in the liver (181). These results

provide evidence that it may be possible to use hepatocyte transplantation techniques to manage hereditary tyrosinemia and, perhaps, other metabolic liver diseases in which the defect is cell autonomous. For example, α_1-AT deficiency involves a cell-autonomous defect and would be an excellent candidate for this strategy.

Genetic Counseling

Restriction fragment length polymorphisms detected with synthetic oligonucleotide probes (184) and family studies (185) allow prenatal diagnosis of α_1-AT deficiency. Nevertheless, it is not clear how prenatal diagnosis of this deficiency should be used and how families should be counseled about the diagnosis. The data reviewed earlier indicate that 85% to 90% of individuals with α_1-AT deficiency do not have evidence of liver disease at 18 years of age and that nonsmoking PIZZ individuals may not develop emphysema or even pulmonary function abnormalities until 60 to 70 years of age. These data could support a counseling strategy in which amniocentesis and abortion are discouraged. The only other data on this subject come from two studies with conflicting results. Results of one study suggested that the incidence of significant liver disease among siblings at risk is 78% (186). The results of the other suggested that the incidence is 21% (11). These studies, however, were retrospective and heavily influenced by bias in ascertainment of patients. The issue will not be resolved until it is studied prospectively, as, for example, in the Swedish population (1).

Population Screening

Results of several studies have suggested that population screening for α_1-AT deficiency would be efficacious. First, there is evidence that knowledge of and counseling about the consequences of α_1-AT deficiency are associated with a reduced rate of smoking among affected adolescents (187,188). Second, although there was initially some evidence for adverse psychological effects (189), more recent results have indicated that there are no significant negative psychosocial consequences in adults who were informed about their deficiency in a follow-up study after neonatal screening in Sweden (190). These data should give new momentum to reconsider screening programs for α_1-AT deficiency.

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Chapter 38
Porphyrias

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Key Concepts

- The porphyrias are metabolic disorders in which abnormalities in heme biosynthesis cause an excessive accumulation and excretion of porphyrins and porphyrin precursors. A defect in an enzymatic step in the heme biosynthetic pathway is present in each of the porphyrias.
- The genes that encode the enzymes of the heme biosynthetic pathway have been cloned and sequenced. This has made it possible to identify the gene mutations that cause the enzyme defects and has shown that each of the porphyrias has genetic heterogeneity (i.e., several different mutations have been found).
- The principal clinical manifestations are photocutaneous lesions, neurologic dysfunction, and structural liver disease. The photocutaneous lesions are caused by the photoactive properties of porphyrins in skin. Neurologic dysfunction, which underlies the acute porphyric attack, is probably caused by a toxic effect of δ-aminolevulinic acid and/or heme deficiency state. Therapy for the acute porphyric attack is designed to stop the factors that precipitate the attack and provide a high carbohydrate diet. Intravenous hematin is administered to restore hepatic heme homeostasis.
- Liver damage in porphyria cutanea tarda is due to the metabolic abnormality and additional factors, such as alcoholism and hepatitis C infection. Phlebotomy is the first line of therapy.
- Liver damage in protoporphyria is caused by the toxic effect of protoporphyrin on the liver. Liver transplantation has been carried out successfully in several patients, but protoporphyrin-induced damage may occur in the new liver.
- Several nonporphyric disorders, particularly those that cause hepatobiliary disease, may be associated with increased urine excretion of coproporphyrin. This is termed *secondary porphyrinuria*. It can usually be distinguished from the acute porphyrias by measuring the urinary excretion of δ-aminolevulinic acid and porphobilinogen (PBG).
- Pseudoporphyria describes a condition with clinical and histologic features similar to porphyria cutanea tarda, but with normal or near normal porphyrin levels.

The porphyrias are metabolic disorders that are characterized biochemically by the excess accumulation and excretion of porphyrins and porphyrin precursors. These compounds are intermediates of the heme biosynthetic pathway, and the elucidation of the biochemical features of the porphyrias follows the delineation of this pathway (1). An important milestone in understanding the pathogenesis of the biochemical abnormalities was in 1970, when a deficiency of porphobilinogen(PBG) deaminase activity

was demonstrated in tissues from patients with acute intermittent porphyria (AIP) (2). Subsequently, an enzyme defect was found for each type of porphyria. During the last decade, the gene mutations that cause the enzyme defects have been identified.

The liver and bone marrow are the major sites of heme production and, therefore, are the principal sites of expression of the biochemical abnormalities. This formed the basis for the early classification of the porphyrias as either hepatic or erythropoietic (3). When the hepatic mixed-function oxidase system is induced by the administration of drugs, the amount of heme synthesized in the liver increases and the rate of formation of the porphyrins and porphyrin precursors increases. Therefore, patients with acute hepatic porphyrias may have substantial variation in the biochemical abnormalities because of various factors that affect the rate of hepatic heme biosynthesis.

The liver also has an important role in the excretion of porphyrins (4). This causes the liver to be susceptible to the toxic effects of porphyrin accumulation. Conversely, hepatobiliary disease of many types causes an increase in the urinary excretion of porphyrins—in particular, coproporphyrin—because excretion of these compounds is diverted from bile to urine. This condition is called *secondary porphyrinuria*.

The principal clinical manifestations of the porphyrias are caused by their effects on the nervous system, skin, and liver. The diagnosis of a specific type of porphyria can be entertained based on the combination of the clinical features. A noteworthy feature of the porphyrias is the relationship between the clinical manifestations and biochemical abnormalities. The porphyrias associated with acute attacks of neurologic dysfunction are characterized by increased accumulation and excretion of the porphyrin precursors δ-aminolevulinic acid (ALA) and PBG. The porphyrias associated with photocutaneous lesions and liver disease are characterized by increased accumulation of the porphyrin compounds themselves.

Heme Metabolism

Heme Biosynthesis

Heme is a member of a group of compounds called *tetrapyrroles*. These compounds are composed of four pyrrole rings arranged into a larger ring by one-carbon bridges (Fig. 38.1). The four pyrrole nitrogen atoms are oriented toward the center of the ring. Because of the central cavity and the chemical properties of the central nitrogen atoms, tetrapyrroles have excellent metal-binding characteristics. Their complexes with iron (heme), magnesium (chlorophyll), and cobalt (vitamin B_{12}) are crucial to living organisms.

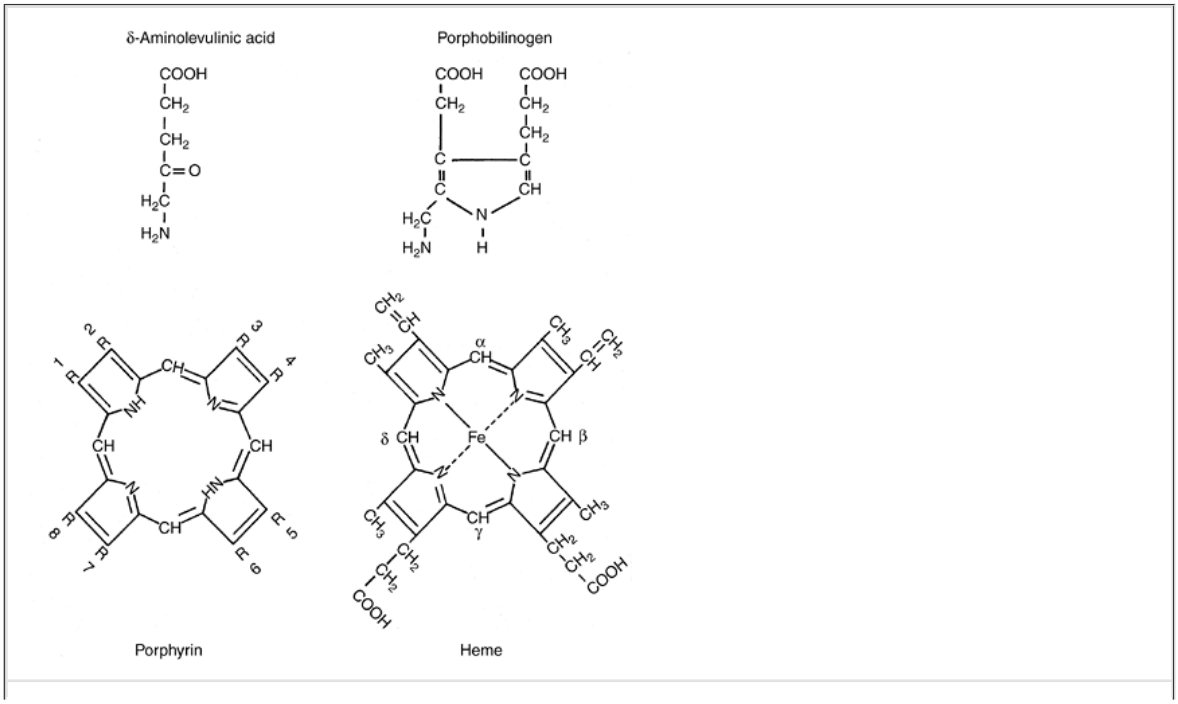
The iron in heme has four of its six coordination positions occupied by the four tetrapyrrole nitrogen atoms. The remaining two coordination positions may be occupied by heteroatoms on the side chains of proteins or by solvent or solute molecules, which, in turn, affect the chemical properties of the iron. The chemistry of the heme moiety is therefore dictated by its protein microenvironment and the nature of the fifth and sixth ligands to iron. Hemoproteins have a variety of chemical interactions, including oxidation–reduction reactions, activation of oxygen, and ligand binding (e.g., oxygen transport). Hepatic hemoproteins include mixed-function oxidases (e.g., cytochrome P-450), dioxygenases (e.g., prostaglandin cyclo-oxygenase), catalase, peroxidases, and tryptophan pyrrolase, all of which are involved in the modification or catabolism of endogenous substrates or potentially toxic compounds. Heme also serves as the oxidation–reduction center for the cytochromes of mitochondrial electron transport and the smooth endoplasmic reticulum.

Heme is synthesized in mammalian tissues in eight steps that are under enzymatic control (Fig. 38.2). In the first step, ALA is formed by the condensation of succinyl-CoA with glycine (5,6). The reaction is catalyzed by the enzyme ALA synthase and occurs in the mitochondrial matrix. Pyridoxal phosphate is required as a cofactor.

ALA diffuses into the cytoplasm of the cell, where two molecules are condensed in a side-to-side manner by the action of ALA dehydrase (7). The product is the monopyrrole PBG. Four molecules of PBG are then joined head to tail, with the displacement of four amino groups through a reaction catalyzed by PBG deaminase, forming the linear tetrapyrrole hydroxymethylbilane (8,9). Dipyrromethane, which is made from PBG by the same enzyme, is a critical cofactor in the reaction. Hydroxymethylbilane spontaneously forms the cyclic compound, uroporphyrinogen I. Porphyrinogens are structures comprising four pyrrole units joined by methylene ($-CH_2-$) groups; they are nonplanar and nonaromatic. Each pyrrole unit of uroporphyrinogen I has an acetate and a propionate side chain that alternate strictly. Enzymic formation of cyclic hydroxymethylbilane is catalyzed by uroporphyrinogen III synthase (also called *cosynthase*) (10,11). This alters the symmetry by reversing the sense of the last pyrrole unit (the D ring). The order of side chains after enzymic cyclization beginning with group R1 (Fig. 38.1) is acetate–propionate (A ring), acetate–propionate (B ring), acetate–propionate (C ring), propionate–acetate (D ring).

Uroporphyrinogen decarboxylase (uroporphyrinogen carboxylase) is a cytosolic enzyme that catalyzes the stepwise decarboxylation of each of the acetate side chains of uroporphyrinogen, leaving methyl substituents (12,13). It is a unique decarboxylating

enzyme in that no cofactors or coenzymes are involved. The enzyme is active with both uroporphyrinogen I and III but not with the oxidized form of the substrate, uroporphyrin. During the course of the decarboxylation, intermediates having seven, six, and five carboxyl groups are generated and are referred to as hepta-, hexa- and pentacarboxylate porphyrinogens, respectively. The final product of the reaction is the tetracarboxylate coproporphyrinogen.



▪ **Figure 38.1** Structures of the porphyrin precursors δ-aminolevulinic acid, porphobilinogen (PBG), porphyrin, and heme. All porphyrins have the same tetrapyrrole ring structure, but they differ in the composition of side chains (R1 to 8) attached to the ring. Uroporphyrin has eight carboxylic acid side chains, coproporphyrin has four, and protoporphyrin has two. Porphyrinogens are the reduced forms of the porphyrins, in which the methene bridges linking the four pyrrole groups are replaced by methylene groups.

The next step is catalyzed by coproporphyrinogen oxidase. The propionate side chains at R2 and R4 of coproporphyrinogen III are oxidatively decarboxylated, forming the vinyl ($-CH=CH_2$) substituents of protoporphyrinogen IX (14). The enzyme, which is located between the outer and inner mitochondrial membranes (15), is active only with coproporphyrinogen III; no other isomer serves as substrate.

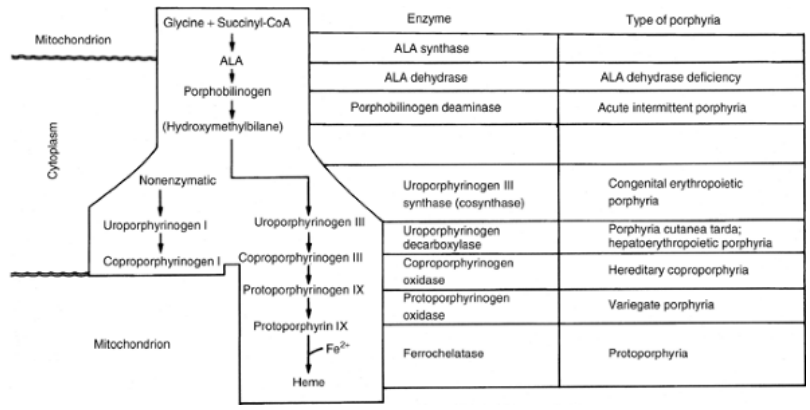
Protoporphyrinogen IX undergoes a six-electron oxidation catalyzed by protoporphyrinogen oxidase (16). In mammalian liver, the reaction requires molecular oxygen as the final electron acceptor, but the primary electron acceptor and electron pathway to oxygen are unknown. The enzyme is located in the inner mitochondrial membrane (17).

The final step in heme biosynthesis involves the insertion of divalent (ferrous) iron into protoporphyrin IX, forming heme; the reaction is catalyzed by ferrochelatase (18), which is located on the matrix side of the inner mitochondrial membrane (19). Human ferrochelatase contains a [2Fe–2S] cluster that is essential for activity (20). The enzyme is active with other dicarboxylic porphyrin substrates and with other divalent metal ions, such as Co^{2+} and Zn^{2+}; it is not active with trivalent metals [e.g., Fe^{3+} or Co^{3+}]. Its functional state is a homodimer, as revealed by its crystal structure (21).

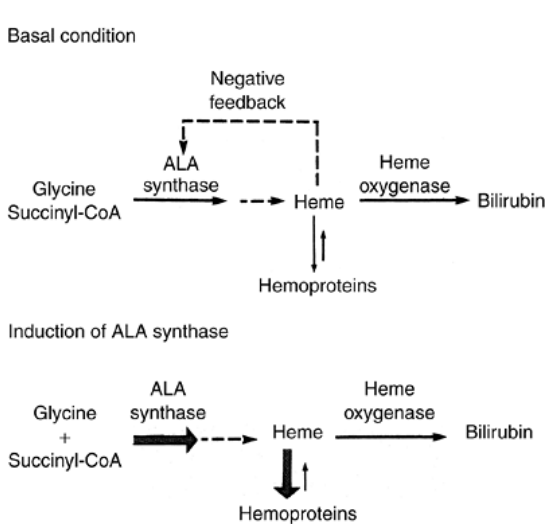
Hepatic Heme Metabolism

The synthesis of heme in liver is controlled primarily by the level of ALA synthase activity, which is, in turn, controlled by a regulatory heme pool through negative feedback inhibition (Fig. 38.3). Two forms of ALA synthase—erythroid and nonerythroid—are encoded by different genes (22). The nonerythroid or housekeeping form of the enzyme, which is present

in the liver, is induced by a variety of chemicals. Heme controls hepatic ALA synthase activity by repressing the synthesis of the enzyme and also by inhibiting its transport from the cytoplasm into mitochondria (23,24). The synthesis is repressed by a decrease in the level of ALA synthase messenger ribonucleic acid (mRNA) (25,26). A heme regulatory motif in the enzyme has been implicated in the inhibition of its transport into mitochondria (27).



▪ **Figure 38.2** Heme biosynthetic pathway, illustrating the enzyme abnormalities that characterize the different types of porphyria. Heme biosynthesis is distributed between the mitochondria and cytoplasm of the cell, as shown. ALA, δ-aminolevulinic acid.



▪ **Figure 38.3** Control of hepatic heme biosynthesis. Under basal conditions, the rate-limiting enzyme in the pathway, δ-aminolevulinic acid (ALA) synthase, is under negative feedback control by a putative regulatory heme pool. An increased demand for hepatic heme biosynthesis, as occurs when there is formation of more cytochrome P-450 or other hemoproteins, may deplete this pool. This removes the negative feedback control and causes induction of ALA synthase and thereby increases the synthesis of porphyrins and porphyrin precursors.

It is estimated that, in humans, the liver accounts for approximately 15% to 20% of the total body production of heme (28). Most heme produced in the hepatocyte enters the hepatic hemoprotein pool. Because heme is formed at the inner mitochondrial membrane, it must be distributed to apoproteins throughout the hepatocyte for hemoprotein activity to be constituted. A heme-binding protein in liver cytosol, which appears to be identical to the liver fatty-acid–binding protein, may have a role in the efflux of heme from mitochondria (29). The microsomal cytochrome P-450 system utilizes most of the hepatic heme, accounting for more than 60%. Other hemoproteins—such as cytochrome b_5, the mitochondrial cytochromes, and catalase—also have critical functions in the liver.

Heme is catabolized to bilirubin through the combined actions of microsomal heme oxygenase and biliverdin reductase (30,31,32). Heme oxygenase is found in both hepatocytes and Kupffer cells, the latter having high activity. Heme oxygenase opens the heme ring, releasing carbon monoxide and iron in the process, to form the green pigment biliverdin. Biliverdin is then reduced by biliverdin reductase to form bilirubin.

Because the hepatocyte has heme oxygenase activity, it can degrade the heme that it produces. Most of the heme that is degraded comes from the functional hemoprotein pool, but a portion of newly formed heme is catabolized to bilirubin before it is incorporated into hemoproteins (33). Some hepatic heme is degraded

through a pathway that does not form carbon monoxide and bilirubin (34).

Hepatic heme oxygenase is a heat-shock protein that is induced by stimuli that cause oxidative stress (30,35), suggesting that it may have a role in the response to hepatic injury. Heme oxygenase may additionally modulate hepatic vascular perfusion through the production of carbon monoxide (36), which causes smooth muscle relaxation.

Biliary Excretion of Heme

Isolated hepatocytes secrete newly synthesized heme into the culture medium (37). There is no evidence that heme synthesized in the liver of the intact organism is secreted into the blood, but studies in the bile fistula of animals and humans indicate that heme is excreted in bile (38,39).

Heme excreted in bile does not enter the enterohepatic circulation because heme is catabolized by the mucosal cells of the intestine. In healthy subjects, the fractional absorption of radioactive iron from food containing inorganic iron salts is 1%, compared with 16% from food containing iron in hemoglobin (40). Microsomal heme oxygenase activity in the intestinal mucosa is at a level similar to that in the liver and spleen; this increases significantly in animals made iron deficient (40). Therefore, heme excreted in bile is absorbed by mucosal cells in the intestine, where heme oxygenase catalyzes its cleavage to release inorganic iron for use in the body.

Excretion of Porphyrins and Porphyrin Precursors

The excretory routes of porphyrins and porphyrin precursors are determined primarily by the solubility of the compounds: Water-soluble compounds are excreted in the urine, whereas water-insoluble compounds are excreted in bile. The porphyrin precursors ALA and PBG are soluble in aqueous solution and are excreted in urine. Uroporphyrin is also water soluble because of the presence of eight carboxyl groups and is excreted

predominately in urine. Protoporphyrin, which has only two carboxyl groups, is poorly water soluble and is excreted almost entirely in bile. Coproporphyrin has four carboxyl groups and is excreted in both bile and urine, with the symmetric coproporphyrin I isomer being preferentially excreted in bile. When hepatobiliary disease occurs, biliary excretion of coproporphyrin diminishes and urinary excretion increases. Therefore, hepatobiliary disease is a cause of increased coproporphyrin excretion in the urine, a condition termed *secondary porphyrinuria*. The porphyrinogens are excreted in a pattern similar to their corresponding porphyrins, except that coproporphyrinogen is excreted in a proportionately greater amount in urine than is coproporphyrin. Unlike bilirubin, none of the porphyrinogens or porphyrins is conjugated before excretion.

In healthy individuals, the predominant porphyrin in bile is coproporphyrin, with small amounts of protoporphyrin and negligible amounts of uroporphyrin (41,42). Protoporphyrin and other 2-, 3-carboxyl porphyrins are predominant in feces. Therefore, most of the protoporphyrin in normal stool is probably derived from bacterial metabolism and ingested food (43).

The mechanism by which the liver excretes protoporphyrin into bile has been investigated extensively in isolated perfused rat liver (44,45,46). In this system, the uptake of protoporphyrin from the perfusing medium occurs by simple or facilitated diffusion and continues at a significant rate, even when excretion into bile is impaired. Intracellular transport is not inhibited by colchicine or monensin, indicating that nonvesicular carriers are targeted to the canalicular membrane. Bile acids facilitate the excretion of protoporphyrin into bile, primarily by increasing the concentration of protoporphyrin that can be attained rather than by increasing bile flow. This is related to the structure of the bile acid. Cholate increases the biliary excretion of protoporphyrin more than that of chenodeoxycholate, which, in turn, has a greater effect than ursodeoxycholate.

Secretion of protoporphyrin into bile appears to be mechanistically linked to the secretion of phospholipid (46). An mdr P-glycoprotein is essential for biliary phospholipid excretion (47), translocating phospholipid from the inner to the outer canalicular membrane leaflet. Protoporphyrin secretion into bile after a protoporphyrin load is reduced by 90% in mice that are homozygous for disruption of the mdr2 P-glycoprotein (48).

Biochemical Abnormalities in the Porphyrias

Enzyme Defects

Each of the eight types of porphyrias is associated with an enzyme defect in the heme biosynthetic pathway that produces a characteristic pattern of abnormal accumulation and excretion of porphyrins and/or porphyrin precursors (Fig. 38.2; Table 38.1) (2,49,50,51,52,53,54,55,56,57,58,59,60,61,62). For example, the deficiency of PBG deaminase activity in AIP causes the excess accumulation and excretion of ALA and PBG, whereas a deficiency of ferrochelatase activity in protoporphyria causes the excess accumulation and excretion of protoporphyrin. The diagnosis of a specific porphyria is made by documenting the pattern of abnormal porphyrin and/or porphyrin precursor

excretion that is characteristic for that type of porphyria. In some types, the diagnosis may be confirmed by demonstrating a deficiency of activity for the enzyme that leads to the biochemical abnormality. This has been most effectively applied to the measurement of erythrocyte PBG deaminase activity to diagnose AIP (Fig. 38.4) (63,64). However, approximately 5% of patients with AIP have a splicing mutation in which normal erythrocyte PBG deaminase activity is preserved (65).

Table 38.1. Biochemical Features of the Porphyrias

Type of porphyria	Enzyme defect	Chromosome location	Major site of biochemical abnormality	Principal biochemical features
Acute intermittent porphyria	PBG deaminase	11q23.3	Liver	ALA and PBG in urine
Variegate porphyria	Protoporphyrinogen oxidase	1q22	Liver	ALA, PBG, and coproporphyrin in urine; protoporphyrin in feces
Hereditary coproporphyria	Coproporphyrinogen oxidase	3q12	Liver	ALA, PBG, and coproporphyrin in urine; coproporphyrin in feces
ALA dehydrase	ALA dehydrase	9q34	Liver	ALA in urine

deficiency				
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	1p34	Liver	Uroporphyrin in urine; isocoproporphyrin in feces
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase	1p34	Liver and bone marrow	Zinc protoporphyrin in red cells; uroporphyrin in urine; isocoproporphyrin in feces
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	10q25.2 q26.3	Bone marrow	Uroporphyrin in red cells and urine; coproporphyrin in feces
Protoporphyria	Ferrochelatase	18q21.3	Bone marrow (liver variable)	Protoporphyrin in red cells, bile and feces
PBG, porphobilinogen; ALA, δ-aminolevulinic acid.				

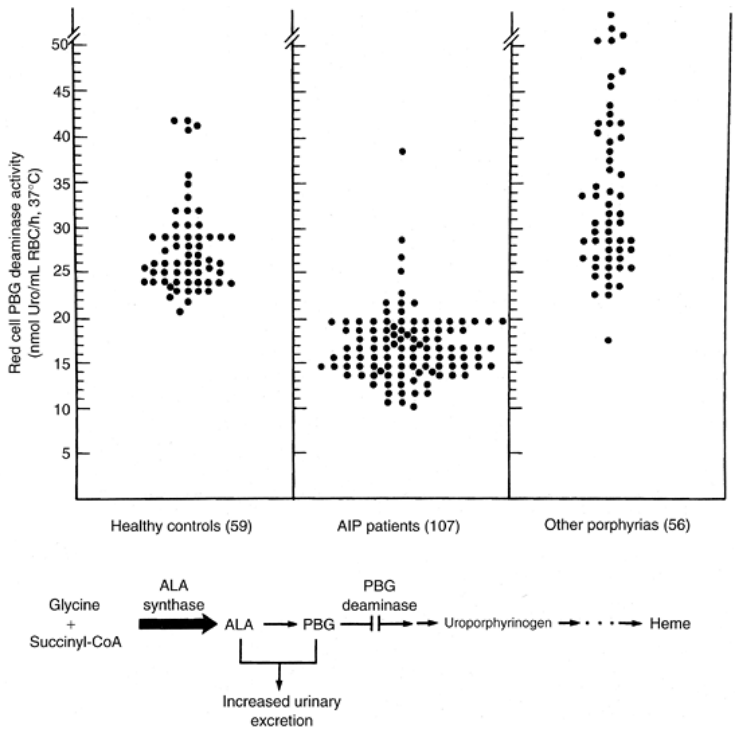
Some of the porphyrias (e.g., AIP, hereditary coproporphyria, and variegate porphyria) have a marked increase in the level of hepatic ALA synthase activity during acute exacerbations of the disease (i.e., acute porphyric attack) (2,66). The increased demand for hepatic heme biosynthesis depletes the regulatory heme pool and thereby removes the negative feedback control on ALA synthase (Fig. 38.3). In AIP, the increased hepatic production of ALA also causes excess formation of PBG. Because of deficient hepatic PBG deaminase activity, ALA and PBG accumulate in greater amounts than they do under basal conditions and are excreted in greater amounts in the urine as well (Fig. 38.4). The acute attack abates when sufficient heme is produced to return hepatic ALA synthase activity to normal, and the excretion of ALA and PBG returns to the basal level. Therefore, patients with AIP may have substantial variation in biochemical abnormalities because of various factors that affect the rate of hepatic heme biosynthesis.

Variegate porphyria is unique in that protoporphyrinogen, which accumulates because of a defect in protoporphyrinogen oxidase, inhibits PBG deaminase (67). This functionally causes a situation same as that in AIP; therefore, excess amounts of ALA and PBG are formed and excreted in the urine when hepatic ALA synthase activity is induced.

Molecular Pathogenesis of Enzyme Defects

The cloning of complementary deoxyribonucleic acid (cDNA) and genes that encode the enzymes of the heme biosynthetic pathway has made it possible to identify the gene mutations that cause enzyme defects; AIP has been the most intensively studied. PBG deaminase activity is usually reduced by approximately 50% in all tissues of patients with this disorder, although for some families the enzyme deficiency is restricted to nonerythropoietic tissues because mutations in exon I selectively affect the nonerythroid form of the enzyme (68,69). More than 200 different mutations have been reported for AIP, demonstrating significant genetic heterogeneity (70,71). These result either in the absence of the protein encoded by the mutant allele or in the synthesis of the protein that has abnormal catalytic properties. The mutations produce abnormal splicing of PBG deaminase mRNA, insertions or deletions in exons that cause premature termination of protein synthesis, nucleotide changes producing stop codons that prevent complete translation of PBG deaminase mRNA, and nucleotide changes causing the substitution of

an amino acid that affects catalytic activity (70). The prevalence of the different mutations is highly variable among different populations. In the United States, no specific mutation has been predominant, whereas in Sweden, nearly half of the families share the same mutation (72).



▪ **Figure 38.4** Enzyme defect in acute intermittent porphyria (AIP). (*Top*) Porphobilinogen (PBG) deaminase activity is deficient in tissues of patients with AIP compared with healthy controls and patients with other types of porphyria. (*Bottom*) As a consequence of deficient PBG deaminase activity, patients with AIP excrete increased amounts of δ-aminolevulinic acid (ALA) and PBG in their urine, particularly when ALA synthase is induced. RBC, red blood cell; nmol Uro/mL, nmol uroporphyrinogen formed per mL red cells. (From Pierach CA, Weimer MK, Cardinal RA, et al. Red blood cell porphobilinogen deaminase in the evaluation of acute intermittent porphyria. *JAMA* 1987;60:257; with permission.)

Genetic heterogeneity has also been found in other types of porphyria. This includes the rare types, such as ALA dehydrase deficiency. The mechanism for variegate porphyria among South Africans is unique in that more than 90% of patients with the disorder have the same mutation in the protoporphyrinogen oxidase gene (73). This strongly supports the founder hypothesis that has been proposed for variegate porphyria in South Africa.

Sporadic porphyria cutanea tarda (PCT) may be an exception to the "rule" that gene mutations are responsible for deficient enzyme activity. Mutations in uroporphyrinogen decarboxylase cDNA or the promoter region of the uroporphyrinogen decarboxylase gene have not been identified, which suggests that sporadic PCT is not caused by mutations at the uroporphyrinogen decarboxylase locus (74). If other inherited factors are responsible for the pathogenesis of human sporadic PCT, the findings in animal models of the disorder may be relevant. In these models, the differences in the expression of liver-specific cytochrome P-450 compounds that generate inhibitors of uroporphyrinogen decarboxylase are inherited (75,76). Inheritance of a hemochromatosis gene mutation may also increase the susceptibility for sporadic PCT (77,78).

However, there has been no clear-cut relationship between specific mutations in porphyric disorders and the severity of clinical and biochemical manifestations.

This is not surprising because clinical expression of a disease is quite variable, even among members of a family. It means that identification of a specific mutation in an individual at an early age probably cannot be used to predict the severity of disease expression.

DNA analysis has not become an established method of diagnosing porphyria. Because of genetic heterogeneity, it may not be a practical way to establish the diagnosis initially, except in geographical areas where a specific mutation has a high prevalence. In a patient with a known mutation, however, DNA analysis should be the method of choice for evaluating family members (71).

The Acute Porphyrias

Three of the porphyrias—AIP, variegate porphyria, and hereditary coproporphyria—are characterized by episodic attacks of neurologic dysfunction, the so-called acute porphyric attack (Table 38.2). These are inherited as autosomal dominant disorders. Porphyric attacks also occur in ALA dehydrase deficiency porphyria, which is inherited as an autosomal recessive disorder (See "Rare Types of Porphyria").

In 1955, Berger and Goldberg proposed the name *hereditary coproporphyria* for a disorder characterized by

acute episodes of neurologic dysfunction accompanied by elevation of urinary ALA and PBG levels. Unlike patients with the previously described porphyrias, these patients had a marked increase in the urinary and fecal excretion of coproporphyrin (88,89). They also had dermatologic lesions. The disorder is much less common than AIP.

Table 38.2. Clinical Features of the Porphyrias

Type of porphyria	Usual inheritance	Neurologic dysfunction	Photocutaneous lesions	Structural liver disease	Hepatocellular carcinoma
Acute intermittent porphyria	AD	+	-	-	+
Variegate porphyria	AD	+	+	-	+
Hereditary coproporphyria	AD	+	+	-	-
ALA dehydrase deficiency	AR	+	-	-	-
Porphyria cutanea tarda	AD (familial type)	-	+	+	+
Hepatoerythropoietic porphyria	AR	-	+	±	-
Congenital erythropoietic porphyria	AR	-	+	-	-
Protoporphyria	AD	-	+	+	-

ALA, δ-aminolevulinic acid; AD, autosomal dominant; AR, autosomal recessive; +, present; –, absent.

Clinical Features of the Acute Porphyric Attack

The clinical features of the porphyric attack are similar for each of the acute porphyrias (Table 38.3) (90), although they tend to be worse in AIP. The severity of an episode depends in part on how much neurologic damage occurred before appropriate intervention was instituted. Women more frequently have acute attacks and appear to have more severe attacks. The episodes may be precipitated by the use of medications (Table 38.4) and by periods of fasting. For some women, attacks occur regularly just before menses, suggesting the importance of female hormones. However, pregnancy is well tolerated by most patients (91).

Abdominal pain is nearly always present. It is caused by autonomic nerve dysfunction. The pain is colicky in nature and often localized to the lower quadrants. An abdominal examination reveals decreased or absent bowel sounds, and abdominal x-rays show alternating areas of spasm and dilatation in the bowel. Pain is relieved by ganglionic blockade; a postmortem study showed destruction of visceral nerve myelin sheaths (92,93). The patients also complain of nausea, vomiting, and constipation or, more rarely, diarrhea. Because there is often an associated leukocytosis, the patient may have to undergo laparotomy for the evaluation of intra-abdominal infection before the diagnosis can be established (90).

Other signs and symptoms of autonomic dysfunction include tachycardia and labile hypertension. When these are present, the patient should be monitored carefully because sudden death has been reported (94,95). Fever, bladder distension, disturbed sweating, and postural hypotension may also occur (90,96).

The peripheral nervous system has both motor and sensory dysfunction. Motor damage occurs early and involves proximal muscle groups; unlike the Guillain Barré syndrome, it tends to involve the upper extremities first (90,96). Electrophysiologic findings indicate an axonal polyradiculopathy or neuropathy (97). Respiratory paralysis can be life threatening, necessitating intubation with ventilator support. Fortunately, this complication usually occurs late in an attack. Diffuse pain involving the extremities, chest, and back is common. Patients also complain of dysesthesias and paresthesias. Deep tendon reflexes are normal at first but are lost progressively in

prolonged attacks. Of note, the ankle reflexes may be selectively preserved.

Central nervous system involvement is also common (90,96). Increased irritability may be the first indication of an impending attack. Insomnia, anxiety, and behavioral changes develop as the attack continues. The patient may become violent and may be labeled as being hysterical, which, in turn, may lead to taking medications that exacerbate the attack. A psychiatric evaluation may reveal severe depression or paranoia; frank psychosis and hallucinations are also seen. Following the acute attack, chronic psychiatric disorders—especially depression—are seen more frequently than in the general population.

Seizures can occur during an acute attack, presenting a difficult clinical problem (90,98,99). Most of the common antiepileptics have a potential for exacerbating the attack. Gabapentin appears to be an exception, presumably because it is not metabolized appreciably

by the liver in humans (100). Inappropriate secretion of antidiuretic hormone with concomitant hyponatremia has been observed, and hypothalamic lesions have been found at necropsy (101,102). Abnormalities in electroencephalograms can occur in the absence of seizure activity, with nonspecific slowing being the most common. Progressive somnolence and eventual coma develop during the advanced attack. The mortality for patients with AIP who required hospitalization was three times that of the general population during the last 50 years, with most deaths occurring during a porphyric attack (103). Survival appears to have improved since the advent of hematin therapy in 1971 (103,104).

Table 38.3. Signs and Symptoms in Acute Porphyric Attacks

Signs and symptoms	Occurrence	%
Autonomic neuropathy	Abdominal pain	95
	Tachycardia (>100 bpm at rest)	80
	Constipation	48
	Nausea, vomiting	43
	Labile hypertension	36
	Postural hypotension	21
	Bladder distension	12
	Dyshidrosis	12
	Fever	9
	Fecal impaction	6
Peripheral neuropathy	Peripheral motor deficit (including respiratory)	60
	Extremity pain, paresthesias	50
	Back pain	29
	Absent reflexes	29
	Chest pain	12
Central nervous system involvement	Bulbar neuropathy	46
	Mental confusion/hallucinations	40
	Seizures	20
	Coma	10

bpm, beats per minute.
From Stein JA, Tschudy DP. Acute intermittent porphyria. A clinical and biochemical study of 46 patients. *Medicine* 1970;49:1, with permission.

Table 38.4. Drugs and the Porphyrias

Drug	Unsafe	Thought to be safe
Analgesics	Antipyrine, oxycodone, pentazocine, phenacetin	Acetaminophen, aspirin, codeine, diflunisal, fenoprofen, fentanyl, ibuprofen, indomethacin, methadone, morphine, sulindac
Anesthetics	Chloroform, enflurane, halothane, isoflurane, lignocaine, prilocaine	Cyclopropane, ether, nitrous oxide, propofol, procaine, succinylcholine

Anticonvulsants	See text	See text
Antimicrobials	Chloramphenicol, dapsone, erythromycin, griseofulvin, ketoconazole, miconazole, nitrofurantoin, rifampin, sulfonamides, trimethoprim, doxycycline (Vibramycin)	Acyclovir, aminoglycosides, amoxicillin, amphotericin, ampicillin, ciprofloxacin, flucytosine, gentamicin, norfloxacin, ofloxacin, penicillin, streptomycin, ticarcillin, vancomycin, zidovudine
Cardiovascular drugs	Amiodarone, nifedipine, simvastatin, verapamil	Adrenaline, atropine, clofibrate, digoxin, heparin, procainamide, quinidine, warfarin
Diuretics and antihypertensives	α-Methyldopa, captopril, clonidine, enalapril, furosemide, hydralazine, hydrochlorothiazide, lisinopril, spironolactone	Acetazolimide, amiloride, bumetamide, ethacrynic acid, guanethidine, labetalol, metoprolol, propanolol, reserpine, timolol, tolazoline
Sedatives and tranquilizers	Alprazolam, amitriptyline, carisoprodol, chlordiazepoxide, diazepam, flurazepam, glutethimide, hydroxyzine, imipramine, loxapine, meprobamate	Chloral hydrate, chlorpromazine, droperidol, prochlorperazine, triazolam
Others	Aminophylline, baclofen, bromocriptine, busulphan, chlorpropamide, cyclosporine, danazol, diclofenac, ergot compounds, glipizide, methotrexate, metoclopramide, tamoxifen, theophylline, tolbutamide	Beclomethasone, chlorpheniramine, colchicine, dexamethasone, famotidine, glucagon, insulin, lithium, quinine

Moore MR, Hift RJ. Drugs in the acute porphyrias—toxicogenetic diseases. *Cell Mol Biol (Noisy-le-grand)* 1997;43:89.

Biochemical Evaluation

For the undiagnosed patient who presents with signs and symptoms of an acute porphyric attack, it is not critical to identify the specific type of porphyria because therapy is the same for all. The urinary excretion of ALA and PBG should be quantitated because excretion of these compounds is increased in acute porphyrias during the porphyric attack. Sodium carbonate (4 g) should be added to the urine collection bottle to prevent degradation of PBG. Four screening tests can be used to detect increased PBG levels in the urine while proceeding with the quantitative measurement: The Watson-Schwartz test, the Hoesch test, the Mauzerall-Granick test, and the Trace PBG kit (Thermo Trace/DMA, Arlington, Texas) (105,106,107). All the tests rely on the reaction of PBG with Erlich's reagent in an acidified solution to form a red compound. An expert panel recently recommended the Trace PBG kit, which detects urine PBG concentrations greater than 6 mg/L, to screen for acute porphyria attacks (107). Because urobilinogen also reacts with Erlich's reagent to form a red compound, particular care must be taken in the Watson-Schwartz test to extract the solution with organic solvents so that a false-positive result is avoided. When allowed to stand in the light and air, urine containing excess PBG may also turn black because of the conversion of PBG to porphobilin and other pigments. In a critically ill patient, these screening studies can be used as a basis for beginning therapy.

After the diagnosis of acute porphyria is made, the specific type of porphyria can be established by measuring erythrocyte PBG deaminase activity (e.g., AIP), the fecal excretion of protoporphyrin and plasma porphyrin fluorescence pattern (e.g., variegate porphyria), and fecal and urinary coproporphyrin excretion (e.g., hereditary coproporphyria).

Pathogenesis of the Acute Porphyric Attack

The mechanism of the neurologic dysfunction that underlies the acute porphyric attack remains unclear. Several factors may be responsible. The development of mice that are made PBG deaminase deficient by gene targeting may provide a means of sorting out the different possibilities (105,106,107,108).

A central role for ALA in the development of the acute attack has been postulated (Fig. 38.5). The level of this compound is increased in blood and urine during an attack of any acute porphyria and normal in the porphyrias

without neurologic crises. Also, in hereditary tyrosinemia and lead intoxication, a marked elevation in ALA level is associated with neurologic features that are indistinguishable from those in the acute porphyrias (109,110,111,112).

ALA is structurally similar to γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (113). It is a potent GABA receptor agonist (114,115); the interaction of ALA with this receptor may be responsible for some of the symptoms in the acute attack. Intraventricular injection of ALA in experimental animals results in neurotoxicity, and, at high concentrations, ALA inhibits neural Na^+/K^+-adenosine triphosphatase (ATPase) and leads to the breakdown of membrane anion gradients (115).

Although it is reasonable to speculate that ALA may be a major toxin involved in the neurologic dysfunction of the acute porphyrias, the severity of the attack does not correlate well with serum or urine ALA levels (90). Moreover, high serum and urine levels occur in patients without demonstrable neurologic abnormalities (116). The cerebrospinal fluid may also be devoid of ALA during an acute attack, although the significance of this is uncertain because cerebrospinal fluid levels do not correlate with intracellular neural levels (116). Many of the aforementioned effects of ALA occur at concentrations that are unlikely to exist in tissues during acute porphyric attacks.

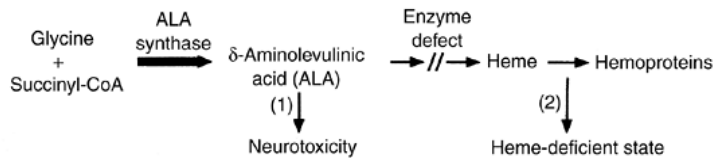
Another potential cause of neurologic dysfunction is impaired heme synthesis. This can produce a decrease in intracellular hemoproteins, leading to depressed cellular respiration in nerve tissue. Impaired hepatic metabolism can also be a consequence of abnormal heme synthesis. A deficiency of hepatic tryptophan pyrrolase, which catalyzes the first step of tryptophan degradation, can result and cause an elevation in the level of 5-hydroxytryptophan (serotonin). Increased excretion of serotonin has been reported in a few patients with acute porphyria (117). Limited heme synthesis in PBG deaminase–deficient mice produces a specific deficiency in the hemoprotein cytochrome P-450 2A5.

Necropsy findings vary with the duration of the attack. If the attack is of short duration, no discernible nerve damage is seen. With prolonged duration, axonal damage and, eventually, demyelinization of autonomic, motor, and sensory nerves occurs (92). This correlates with the observation that early therapeutic intervention (i.e., prior to neuronal death) is associated with complete and rapid recovery. Once paralysis is established, recovery depends on axonal regeneration. Recovery of proximal muscle groups occurs earlier than it does in the distal groups, which are innervated with longer axons. PBG deaminase–deficient mice develop a progressive motor neuropathy with normal or minimally elevated ALA levels (106). These data are most consistent with a dysfunction of hemoproteins causing the neuropathy of porphyria (106).

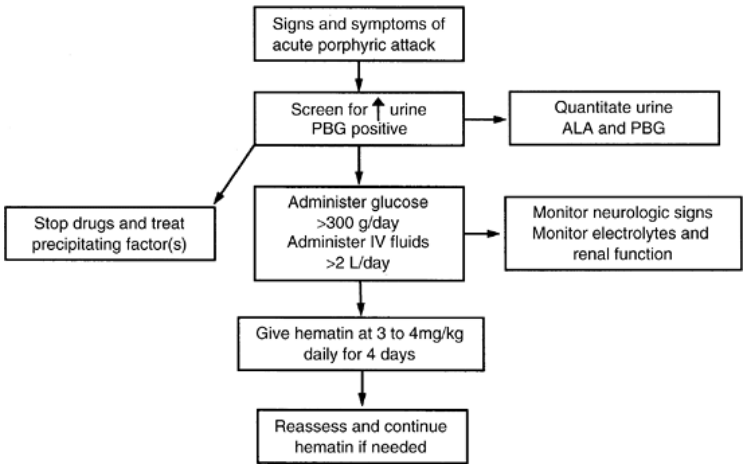
Management of the Attack

Because of the unpredictable nature of an acute porphyric attack, it is generally advisable to hospitalize the patient early during an episode. Therapy should follow several steps (Fig. 38.6). First, any porphyrinogenic drug should be discontinued, and drugs that may exacerbate the attack should be avoided (Table 38.4). If the attack is precipitated by an infection, the infection should be treated promptly. An adequate caloric intake should be maintained because diminished oral intake can precipitate or aggravate an attack (118,119). The diet should provide at least 400 g of glucose or another rapidly metabolized carbohydrate daily. A high carbohydrate intake is beneficial because of its suppressive effect on hepatic ALA synthase activity (120). Patients who cannot take carbohydrates by mouth should be given a high carbohydrate feeding through a nasogastric feeding tube and/or intravenously as 10% glucose. Intravenous fluids should be given at a rate more than 2 L/day in the form of normal saline. The patient should

be monitored for the development of hyponatremia, which may occur because of the inappropriate secretion of antidiuretic hormone. The patient should also be monitored for the progression of neuropathy, with particular attention to changes in respiratory function. Serial measurements of vital capacity and forced expiratory volume should be taken, and ventilatory support should be provided if respiratory depression develops.



▪ **Figure 38.5** Schematic showing that neurologic dysfunction in the inducible porphyrias is linked with the biochemical abnormalities. It may be caused by the neurotoxic effect of δ-aminolevulinic acid (ALA), a heme-deficient state, or a combination of both.



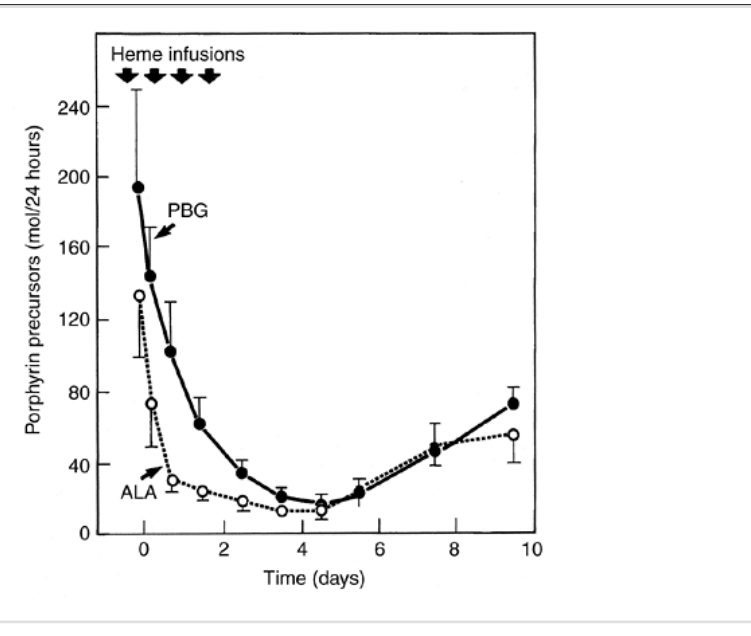
▪ **Figure 38.6** Scheme for the management of acute porphyric attack. ALA, δ-aminolevulinic acid; PBG, porphobilinogen.

Although the product labeling recommends an initial trial of intravenous glucose, an expert panel recommends starting hematin therapy early for most acute porphyric attacks (104). *Hematin* (ferriheme hydroxide) is the chemical form of heme in aqueous solution at physiologic pH. Exogenously administered hematin suppresses hepatic ALA synthase activity (23,121,122,123). Hematin was first administered to a patient with an acute porphyric attack in 1971 (124) and became available as an orphan drug in 1983. It is available in the United States as lyophilized powder for reconstitution into sterile water just before infusion (Panhematin, Ovation Pharmaceuticals, Deerfield, Illinois). In Europe, it is also available as heme arginate (Normosang, Leiras Oy Pharmaceuticals, Helsinki, Finland).

Hematin, administered intravenously in a dose of 3 to 4 mg/kg body weight once daily for 4 days, causes a prompt decline in serum and urine levels of ALA and PBG (Fig. 38.7) (125,126). Antipyrine metabolism also improves (127,128,129,130), suggesting that apoproteins of cytochrome P-450 are reconstituted with heme therapy. Despite its impressive pharmacologic effects, the clinical effect of hematin is less predictable. There have, in fact, been no randomized, double-blind studies that show a clinical benefit for hematin during an acute porphyric attack, although many physicians who manage such patients believe that it is of benefit. Hematin should be given before advanced neuronal damage occurs because this is usually not reversed.

The most common complication of hematin therapy is thrombophlebitis, which can be prevented by

administering the solution for 15 to 30 minutes in a free flow through an intravenous line. A transient coagulopathy may be caused by breakdown products of hematin; therefore, it should be given as soon as possible after it has dissolved in an aqueous solution. Dissolving hematin in human serum albumin protects it from degradation (129). Tin protoporphyrin, which inhibits heme oxygenase and thereby prevents the breakdown of heme, has been used in combination with hematin to prolong biochemical remission (131).



▪ **Figure 38.7** Effect of intravenous infusion of heme arginate (3 mg/kg) on the excretion of δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) in six patients with acute intermittent porphyria (mean ± SE). (From Mustajoki P. Acute intermittent porphyria. *Semin Dermatol* 1986;5:155, with permission.)

The symptoms and signs of the attack should be treated while glucose and/or hematin is given. Pain, which can be very severe, should be controlled with narcotics, if necessary. Agitation can be controlled with chloral hydrate or chlorpromazine, and ondansetron can be used to relieve nausea and vomiting. Propranolol, in a dosage of 20 to 200 mg/day, can be used for hypertension and tachycardia, although it should be used cautiously (132). Seizure activity is a particularly difficult problem because most of the anticonvulsants (particularly the barbiturates and phenytoin) precipitate acute attacks. Bromides are safe but difficult to use. Clonazepam in low doses that produce levels up to 6 mg/dL may be administered, and new drugs, such as gabapentin, are promising (100). Status epilepticus can be controlled with diazepam (up to 10 mg intravenously), paraldehyde (8 to 10 mL rectally), or magnesium sulfate (0.5 to 1.0 g/hour by intravenous infusion).

Many patients have a limited number of porphyric attacks in their lifetime and do well if they avoid porphyrinogenic drugs, fasting, and excessive alcohol intake. However, some patients have recurrent attacks and pose a challenging problem. They are prone to narcotic addition, and pain management becomes a critical feature of their care. Available studies have not clearly shown that the regular administration of hematin is beneficial in this situation (133). For such patients liver transplantation may be considered because this has corrected the biochemical and clinical abnormalities in one patient with AIP (134).

In some women, attacks are related to the menstrual cycle, beginning a few days before the onset of menses and ending after it has begun. The attacks are probably caused by endogenous progesterone. Analogs of gonadotrophin-releasing hormone have been used in the management of these cases (135). These agents prevent the normal cyclic secretion of luteinizing hormone and follicle stimulating hormone by the pituitary. Oral hormonal contraceptive use is associated with acute attacks in some patients, but menopausal hormone replacement therapy only rarely affects acute porphyries. Pregnancy is usually well tolerated, but there is an increased frequency of miscarriage.

Prevention of Attacks

Individuals who have suffered acute porphyric attacks should wear bracelets stating that they have porphyria. They should also be provided a list of drugs that are safe for use, as well as those that are unsafe. They should be instructed to avoid fasting and excess intake of alcohol and to have infections treated promptly. Treatment with erythropoietin may reduce the severity of porphyric attacks in some patients (136).

Measurement of erythrocyte PBG deaminase activity has shown that many individuals related to patients with AIP are latent carriers of the gene defect. They have no clinical manifestations, and many have normal excretion rates for ALA and PBG. Although the natural history of the latent state remains unclear, carriers of the gene defect have the potential to develop acute attacks and should therefore avoid factors that are incriminated in precipitating attacks (e.g., sulfonamides, barbiturates, hydantoins, fasting, and excessive intake of alcohol).

Erythrocyte PBG deaminase activity should be measured in first-degree relatives of patients with AIP. Children should be assessed as they approach puberty. As gene testing develops, it should also be utilized for families in which acute porphyria is present. Patients in whom the enzyme abnormality/gene defect is found should be managed along the guidelines outlined for latent individuals.

Hepatocellular Carcinoma in Acute Porphyrias

Limited information is available about structural liver changes in the acute porphyrias. Fatty infiltration and siderosis, together with a mild inflammatory process, have been seen in liver biopsy specimens, and crystalline material has been found within mitochondria (134,137). Functional studies have shown there is impaired hepatic mixed-function oxidase activity (137).

There is an association between the acute porphyrias and hepatocellular carcinoma (138,139,140). The relative risk of developing hepatocellular carcinoma in acute porphyrias is 30 to 60 times that for the general population (138,141). It has been speculated that carcinogenic substances may accumulate because the hepatic detoxification mechanism is impaired (140).

Porphyria Cutanea Tarda

PCT is traced to Günther's description, in 1911, of adult patients who had cutaneous lesions but no neurologic abnormalities (142). The term was first used by Waldenström to distinguish the disorder from the

porphyrias in which both cutaneous lesions and neurologic dysfunction occur (80). *Tarda* was included in the name because the disorder occurred late, commonly after the fourth decade of life. However, the familial form of PCT may begin in childhood (143,144).

PCT is the most common porphyria with clinical expression in the United States, although its exact prevalence is unknown. It is frequently associated with ethanol use. Among the Bantu of South Africa, a high prevalence is seen; this has been attributed to the ingestion of a local beer brewed in iron pots (145). The disorder was previously identified much more commonly in men, but the proportion of women diagnosed with PCT has

increased. This reflects, in part, the increasing use of oral contraceptives and estrogen preparations, which also precipitate PCT (146).

Although most cases are sporadic, families with PCT have been identified, in which there is autosomal dominant inheritance with variable penetrance (54,57,147). In contrast to the sporadic cases, in which the abnormality in uroporphyrinogen decarboxylase is restricted to the liver, the familial cases also have a deficiency of erythrocyte uroporphyrinogen decarboxylase activity (148).

PCT can also be acquired through toxic exposure. In the late 1950s, an epidemic occurred in Turkey after the widespread ingestion of seed grain that had been treated with the fungicide hexachlorobenzene (149). Experimental porphyria mimicking PCT has been induced with a number of structurally related compounds, including polychlorinated and polybrominated biphenyl and dioxin compounds (150).

Disorders Associated with Porphyria Cutanea Tarda

Several disorders are associated with the development of PCT. Most notable has been the striking association with chronic hepatitis C (See "Porphyria Cutanea Tarda and Hepatitis C"). Systemic lupus erythematous, rheumatoid arthritis, Sjögren's syndrome, and acquired immunodeficiency syndrome (AIDS) have also been associated with PCT (151,152,153,154,155). Increased porphyrin levels in the dialysate and plasma of patients on long-term hemodialysis have been reported (156,157,158). This may occur irrespective of the development of cutaneous lesions (158).

Porphyria Cutanea Tarda and Hepatitis C

There is a significant increase in the prevalence of chronic hepatitis C in patients with PCT compared with the general population (151,152,153,154,155,159,160,161,162,163,164). There is striking geographic variation in this association. The highest prevalence is found in southern European countries, where chronic hepatitis C is found in 60% to 90% of patients with PCT (152,159). A large study in the United States found the prevalence to be 56% in 70 unselected patients with PCT (78). Hepatitis C virus (HCV) infection was documented by a positive test for both hepatitis C antibody and RNA. The patients with PCT who had hepatitis C infection did not differ from those without infection in terms of the type or severity of skin lesions, or in the level and pattern of urinary porphyrin excretion. However, this condition was more frequent in men and among those with a higher rate of alcohol use.

The causal relationship between hepatitis C and PCT, if any, remains unclear (165). Only about 1% to 5% of patients with chronic hepatitis C have clinical PCT (159), and conversely many patients with PCT do not have chronic hepatitis C. Viral parameters of hepatitis C such as genotype and viral level do not appear to be important (166). Other possibilities are that hepatitis C alters hepatic iron metabolism or promotes oxidative stress in hepatocytes, which brings out overt PCT in susceptible individuals. Overall, PCT is now considered to be an extrahepatic manifestation of HCV infection.

Porphyria Cutanea Tarda and HFE Mutations

Hepatic iron overload is common in patients with overt PCT. Because iron removal returns porphyrin excretion to normal and ameliorates the clinical features in PCT, hepatic iron overload appears to be an important factor in causing the disease. Before the identification of the *HFE* gene, it was controversial whether patients with PCT may have inherited a gene mutation for hemochromatosis, which caused hepatic iron overload. Subsequently, studies from many countries demonstrated that 40% to 50% of patients with PCT carry the C282Y mutation in the *HFE* gene, with 10% to 20% being homozygous (77,166,167). A study from the United States showed that 42% of patients with PCT carried the C282Y mutation (15% were homozygous) and another 31% carried the H63D mutation (8% were homozygous). Therefore, there appears to be an increased prevalence of the hemochromatosis gene mutations in patients with PCT (168), suggesting a mechanism by which some patients with PCT have increased hepatic iron levels.

Biochemical Evaluation of Porphyria Cutanea Tarda

The diagnosis of PCT is generally made on a clinical basis, but biochemical confirmation, by demonstrating increased urine excretion of uroporphyrin, should be made. Urine heptacarboxyl and hexacarboxyl porphyrin levels are also elevated. Coproporphyrin and

pentacarboxyl porphyrin levels are elevated to a lesser degree. Urinary PBG is not increased, but a slight elevation in ALA level is common. Fecal porphyrin analysis demonstrates that porphyrins are present in the form of isocoproporphyrins.

Photocutaneous Lesions in Porphyria Cutanea Tarda

The presenting clinical manifestation of PCT is nearly always the development of bullous lesions in areas of sun exposure (Fig. 38.8). The lesions occur after minor trauma because of increased skin fragility. The dorsum of the hand is most frequently involved. Other sites include the forehead, neck, and ears. The bullae may become infected, causing delayed healing that produces scarring and pigment changes. Hypertrichosis occurs in the periorbital area, a feature that was prominent in the Turkish epidemic. Milia, which are small white papular lesions, form as a chronic manifestation of the disorder. Sclerodermoid changes may also develop.

The skin lesions in PCT are the same as those in variegate porphyria and hereditary coproporphyria. The rare disorder, hepatoerythropoietic porphyria, also features skin lesions similar to those in PCT. In contrast to PCT,

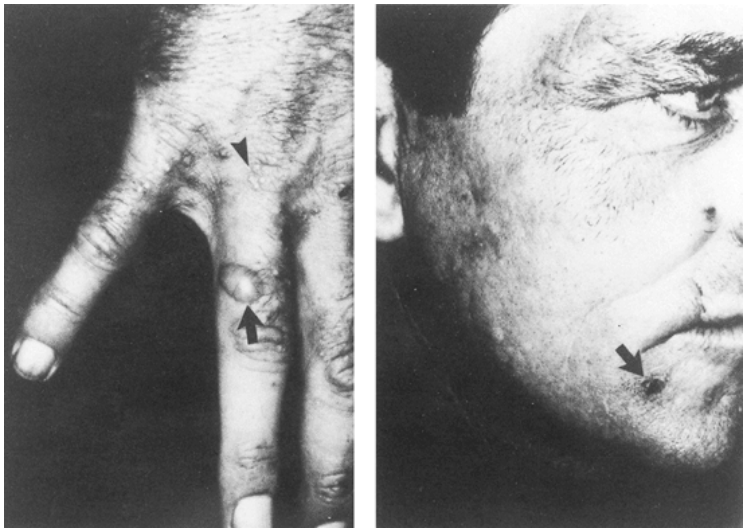
skin lesions occur in infancy and tend to diminish with age.

Porphyrins are produced in excess and accumulate in the skin of patients with the cutaneous porphyrias. In the disorders in which bullous lesions occur, hydrophilic porphyrins accumulate within lysosomes of cells (169). The severe lesions in PCT may be due to the release of proteolytic enzymes from lysosomes into the cytoplasm (Fig. 38.9) (170). Complement activation may also contribute to the pathogenesis of the cutaneous lesions in PCT. Complement compounds are deposited at the dermal–epidermal junction near bullae, and elevated levels of complement components and cleavage products are found in the fluid contained in the lesions (171). Complement levels in serum containing excess porphyrin decline after ultraviolet irradiation (171). Uroporphyrin also stimulates collagen biosynthesis by fibroblasts, which may contribute to the sclerodermoid skin changes in PCT.

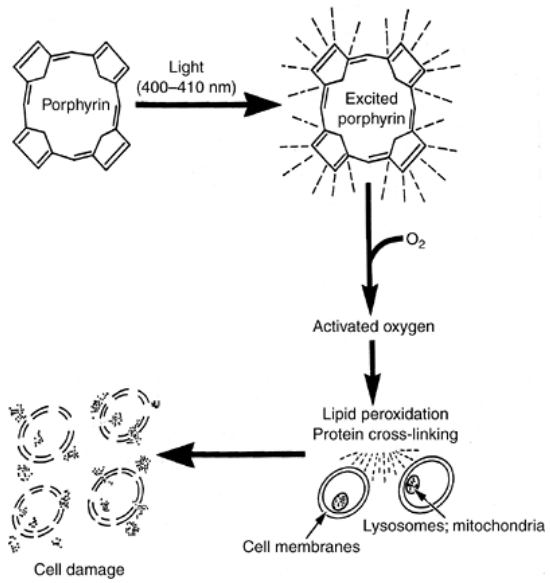
Liver Damage in Porphyria Cutanea Tarda

Patients with clinically overt PCT usually have liver damage. Approximately two thirds of patients have an elevation in the serum transaminase level at the time of diagnosis (172,173,174,175). The liver may have a patchy gray discoloration when viewed grossly (172). Liver biopsy specimens exhibit red fluorescence when exposed to

ultraviolet light (176). If a water-free preparation of tissue is used, a cytoplasmic distribution of fluorescence is observed. Needle-like cytoplasmic inclusions are found in specimens from untreated patients (Fig. 38.10) (177). The inclusions, which appear to be uroporphyrin crystals, are water soluble and are lost during tissue processing unless water-free fixation is used.



▪ **Figure 38.8** Skin lesions in porphyria cutanea tarda. Erosions and bullae (*arrows*) occur in sun-exposed areas after minor trauma. Milia (*arrowhead*) are small, whitish papules found on the dorsal aspects of the hands. There is increased facial hair in the periorbital region. Similar skin changes are found in variegate porphyria, hereditary coproporphyria, and hepatoerythropoietic porphyria. (From Bloomer JR. The hepatic porphyrias. *Gastroenterology* 1976;71:689, with permission.)



▪ **Figure 38.9** Sequence by which damage to skin and other tissues may be produced when porphyrins are exposed to light in the presence of molecular oxygen. Uroporphyrin preferentially causes injury to lysosomes in cells, whereas cell membranes and mitochondria appear to be the principal targets in protoporphyrin-induced damage.

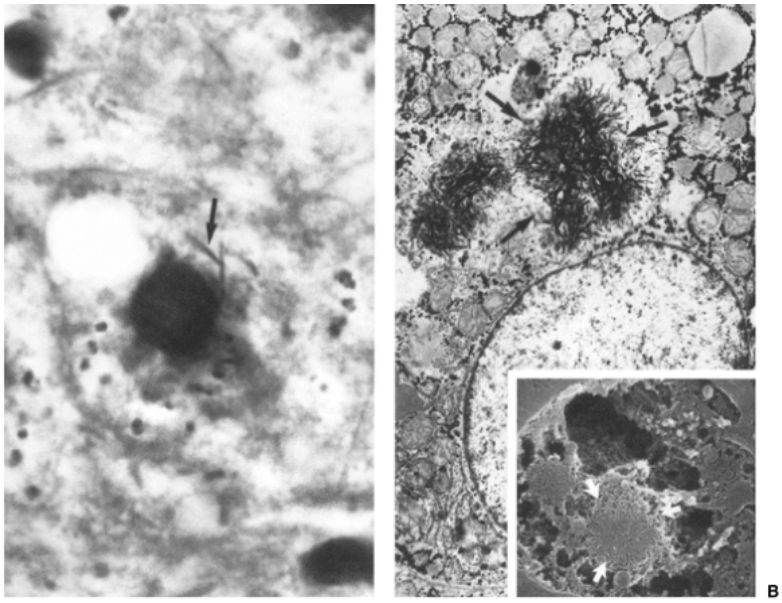
Patients with PCT commonly have some degree of hepatic iron overload, and hemosiderosis is usually demonstrated with iron stains (134,172,174,175). The accumulation of iron occurs not only in patients who abuse ethanol but also in familial and estrogen-induced PCT (134,174). The iron is distributed diffusely but is most frequent in Kupffer cells and clusters of macrophages.

Fatty infiltration of the liver is also common, having been reported in most biopsy specimens. Although this may be related in part to underlying ethanol abuse, one study found no difference in the prevalence of fatty liver in alcoholic versus nonalcoholic patients with PCT (174). The fatty changes are usually mild.

Granuloma-like clusters of mononuclear cells, Kupffer cells, hemosiderin, and ceroid have been described as lobular lesions of PCT (175). They occur with variable frequency and may represent a reaction to collections of iron and uroporphyrin.

Hepatitis C may contribute to the liver damage in PCT. Liver biopsy specimens from patients with PCT who are positive for hepatitis C antibody have changes similar to those from patients with hepatitis C alone, including lymphoid follicles, bile duct damage, and hepatocellular necrosis (153).

The extent of hepatic damage is variable, ranging from minimal injury to cirrhosis, and appears to be related, in part, to the duration of PCT (175). No definite relationship has been established between ethanol abuse and progression of liver damage, and alcoholic hepatitis is infrequent in PCT (172,174). Although hepatic iron overload may contribute to liver damage, the correlation between the degree of siderosis and the severity of liver damage is poor. Nevertheless, phlebotomy produces improvement in some, but not all, of the abnormalities. Hemosiderosis disappears as total iron stores are depleted. Fluorescence and the crystalline material, as well as the granuloma-like lesions, disappear. The liver enzyme abnormalities return toward normal. Improvement usually occurs irrespective of continuation of the use of ethanol (173).



▪ **Figure 38.10** Hepatic crystals in porphyria cutanea tarda and protoporphyria. **A:** Light micrograph shows intracytoplasmic crystals (*arrow*) in a hepatocyte of a patient with porphyria cutanea tarda (hematoxylin and eosin stain). **B:** Transmission electron micrograph shows a cluster of crystals (surrounded by *arrows*) in a hepatocyte of a patient with protoporphyria. A scanning electron micrograph shown in the *inset* also demonstrates the crystalline material (surrounded by *arrows*). (From Rank JM, Straka JG, Bloomer JR. Liver in disorders of porphyrin metabolism. *J Gastroenterol Hepatol* 1990;5:573, with permission.)

PCT has been associated with hepatocellular carcinoma. The first report of this relationship was in 1957, when apparent PCT was noted in a patient with a hepatic tumor that fluoresced when exposed to ultraviolet light (178). After surgical removal of the tumor, the patient's skin lesions improved and porphyrin excretion returned to normal. There have been other reports of porphyrin-excreting tumors in which the biochemical abnormalities were somewhat different from those usually seen in PCT, with increased excretion of several porphyrin compounds in addition to uroporphyrin (179).

The more common association is the development of hepatocellular carcinoma in patients with long-standing PCT. The frequencies of cirrhosis and hepatocellular carcinoma were 63% and 53%, respectively, in a 1979 autopsy series (180). The diagnosis of PCT had been made several years before death in all cases. A 1972 autopsy series of patients with PCT in Czechoslovakia found cirrhosis in 64% and carcinoma in 47% (181). The severity of liver disease and the development of carcinoma correlated with the length of time after the diagnosis of PCT was made. The duration was 3.7 years for patients without cirrhosis, 6.3 years for those with cirrhosis but no carcinoma, and 11.7 years for those with carcinoma. Cirrhosis was present in all cases with hepatocellular carcinoma. Ethanol use was not universal, and the degree of hepatic hemosiderosis was variable. In two other studies, conducted in 1982 and 1985, an increased incidence of hepatocellular carcinoma was also found (182,183). The presence of carcinoma correlated with male sex, duration of time after the diagnosis of PCT, and cirrhosis. There was no correlation with ethanol use or hepatitis B serology. However, none of 96 Italian patients screened with radionuclide liver scan and measurement of serum α-fetoprotein had evidence of tumor (184).

Because these studies were carried out before the discovery of the HCV, the role of chronic hepatitis C in the development of hepatocellular carcinoma could not be determined. A recent study from the Netherlands found the incidence of hepatocellular carcinoma to be 13% in the 38 patients with PCT who were followed up for 2 to 18 years (161). There was no difference in the prevalence of hepatitis C infection in patients with hepatocellular carcinoma (20%) compared to those

without (18%). Therefore, hepatocellular carcinoma may develop in patients with PCT irrespective of the presence of hepatitis C infection.

These studies suggest that there is an increased risk for developing cirrhosis and hepatocellular carcinoma for patients with long-standing PCT. Additional studies are needed to clarify the role of hepatitis C in this process. In any event, it is probably prudent to screen patients with long-standing PCT, particularly those with chronic hepatitis C and/or cirrhosis, for hepatocellular carcinoma by measuring serum α-fetoprotein and by hepatic ultrasonography.

Management in Porphyria Cutanea Tarda

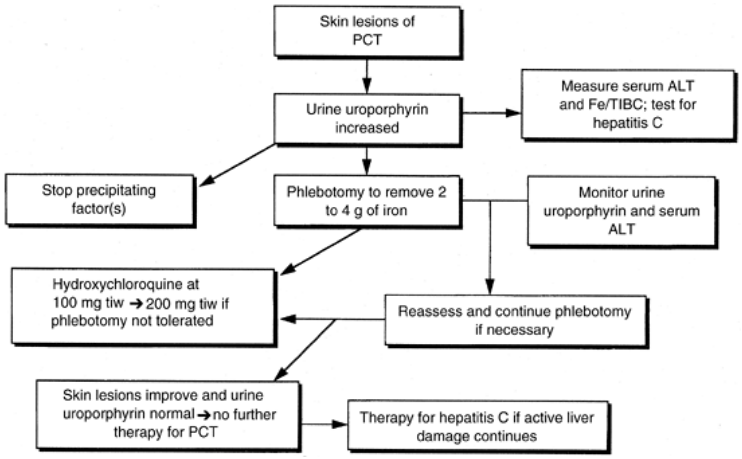
The patient with active PCT should avoid wavelengths of light that may excite porphyrins. This occurs maximally at a wavelength of 400 to 410 nm. Light of this wavelength is not filtered by window glass; therefore, the patient should take precautions when driving a car. Fluorescent lights should also be avoided.

Patients should stop ingesting ethanol, and women taking birth control pills should use another form of contraception. Patients should also not take iron-containing compounds. Despite discontinuing the precipitating factors, improvement will be slow unless other therapy is used (Fig. 38.11). The mainstay of therapy is phlebotomy (185,186), which is based on the observation that hepatic siderosis is common and that iron probably plays an important role in the pathogenesis of the disease. The liver typically contains an excess of 2 to 4 g iron, and the amount of phlebotomy needed will be on the order of 4 to 8 L of blood. After phlebotomy is completed, urine levels of uroporphyrin will continue to decrease toward normal, and more than 90% of patients will have normal levels after 6 to 12 months. Resolution of skin fragility will accompany the decrease in uroporphyrin excretion, and patients will no longer develop vesicles or erosions. Hirsutism and hyperpigmentation may take months to clear after phlebotomy is completed, and sclerodermoid changes may not resolve for several years. Iron-containing compounds should not be ingested because this may cause a relapse.

For patients who do not tolerate phlebotomy or who continue to have cutaneous symptoms despite an adequate course of phlebotomy, chloroquine or related compounds can be administered (186). These compounds appear to form complexes with uroporphyrin and heptacarboxyl porphyrin, enhancing their removal from tissues and excretion in urine. The initial dose should be 100 mg of hydroxychloroquine or 125 mg of chloroquine three times a week. Larger doses may cause hepatic injury related to the massive removal of uroporphyrin from the liver. In an uncontrolled clinical trial, high-dose vitamin E reduced urine uroporphyrin levels in five patients with PCT (187).

It is unclear how the patient with PCT and concomitant chronic hepatitis C should be managed. A few reports suggest that interferon therapy may reduce

the manifestations of PCT. However, until this has been studied more carefully, it is probably best to institute a course of phlebotomy before starting antiviral treatment.



• **Figure 38.11** Scheme for management of the patient with active porphyria cutanea tarda (PCT). ALT, alanine aminotransferase; TIBC, total iron-binding capacity; tiw, three times a week.

For the patient with chronic renal failure who develops cutaneous lesions of PCT while on hemodialysis, standard phlebotomy is usually contraindicated because of anemia. In this situation, erythropoietin therapy accompanied by small-volume phlebotomies has been used successfully (188).

Erythropoietic Protoporphyria

In 1961, Magnus et al. (189) described a 35-year-old man who had lifelong itching and edema of his skin on exposure to sunlight. His urinary excretion of porphyrins and porphyrin precursors was normal, but his red cells and feces contained excess protoporphyrin; therefore, they proposed the name *erythropoietic protoporphyria* (*EPP*) for his condition. *Erythrohepatic protoporphyria* and *protoporphyria* have also been used as names for the disorder.

EPP occurs in all ethnic groups, but the precise prevalence has not been determined for any group. The pattern of inheritance was previously considered to be that of an autosomal dominant disorder with variable expression. Some individuals who carry a mutant ferrochelatase gene in one allele have no clinical manifestations of the disease, and their porphyrin levels may be normal. Most patients with symptomatic protoporphyria have ferrochelatase activity that is significantly less than the expected 50% of normal in a classic autosomal dominant disease (190). These results are explained in part by the inheritance of a mutation in one ferrochelatase allele that structurally alters the protein together with a low-expressing nonmutant ferrochelatase allele caused by a polymorphism in an intron (IVS 3–48 T/C) (191,192). There is no difference between sexes in

the frequency of the disorder, either for the gene defect or for the clinically expressed disease.

Biochemical Evaluation of Erythropoietic Protoporphyria

The biochemical hallmark of EPP is an increased level of protoporphyrin in red cells and feces. The excess protoporphyrin does not form complexes with a metal, unlike iron deficiency and lead poisoning, in which excess red cell protoporphyrin is chelated to zinc (193). The diagnosis is established by demonstrating an elevated red cell protoporphyrin level in a patient who has the typical clinical features.

Patients with EPP do not excrete increased amounts of PBG and ALA in urine. This implies that hepatic ALA synthase activity is not increased, although in vitro measurements have shown otherwise in some instances (194). In most patients normal amounts of heme are synthesized in liver tissue and bone marrow, but in approximately 25% there is mild anemia characterized by microcytic indices (195). Iron metabolism is normal (196), and iron deficiency can exacerbate the accumulation of protoporphyrin in red cells (197). Once the red cell enters the circulation, protoporphyrin is released into the plasma within a few days (unless liver disease is present) and is then excreted by the liver into bile (Fig. 38.12) (See "Excretion of Porphyrins and Porphyrin Precursors") (198).

Bone marrow is the major source of excess protoporphyrin in most patients. Studies using radiolabeled precursors of protoporphyrin have indicated that the liver also contributes to excess protoporphyrin production (199,200), although the interpretation of these studies has been controversial (201). Metabolic balance studies comparing fecal protoporphyrin excretion with the total mass of red cell protoporphyrin have occasionally demonstrated a significant discrepancy, also indicating a hepatic contribution (202).

Photosensitivity in Erythropoietic Protoporphyria

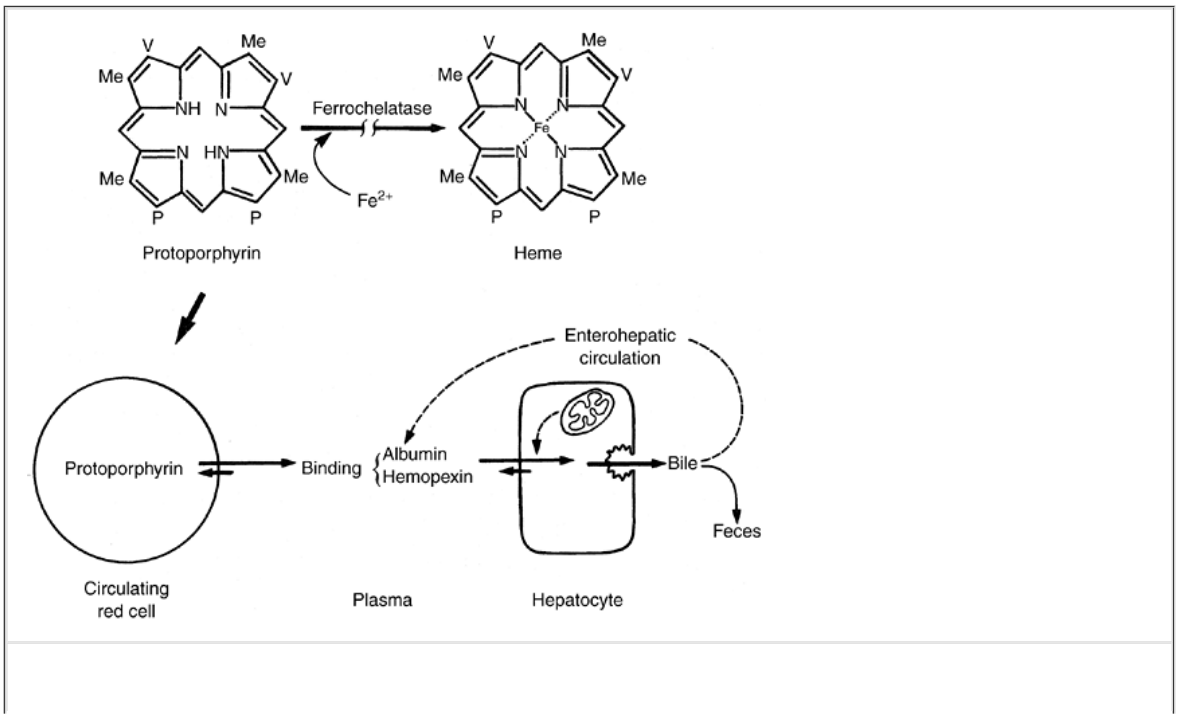
The principal clinical manifestation in EPP is photosensitivity. This is usually lifelong, often beginning in infancy. Rarely, the photosensitivity has onset in adulthood (203). Patients experience burning or stinging of the skin on exposure to sunlight. Window glass does not prevent the reaction because the wavelength of light that causes the photosensitivity (400 to 410 nm) is not filtered by window glass. For some patients, photosensitivity is caused by light emitted from fluorescent fixtures. Erythema and edema of the skin develops and may persist for several days (Fig. 38.13). Unlike PCT, the development of vesicles and erosions is rare. Chronic skin changes are characterized by thickening and lichenification of the skin over the nose and the dorsum of the hand, as well as shallow scars (Fig. 38.13. Microscopic examination of the skin demonstrates the deposition of periodic acid schiff (PAS)-positive material around the walls of capillaries in the dermis (204).

Photosensitivity is caused by excess protoporphyrin circulating in blood or deposited in skin tissue, or a combination of the two. Absorption of light energy by the porphyrin molecule raises the molecule to an excited state in which it reacts with molecular oxygen to produce reactive oxygen species (Fig. 38.9). Cell membranes and mitochondria appear to be the principal targets for protoporphyrin-induced damage (169). The damage is attributed to cross-linking of membrane proteins and peroxidation of membrane lipids (205,206).

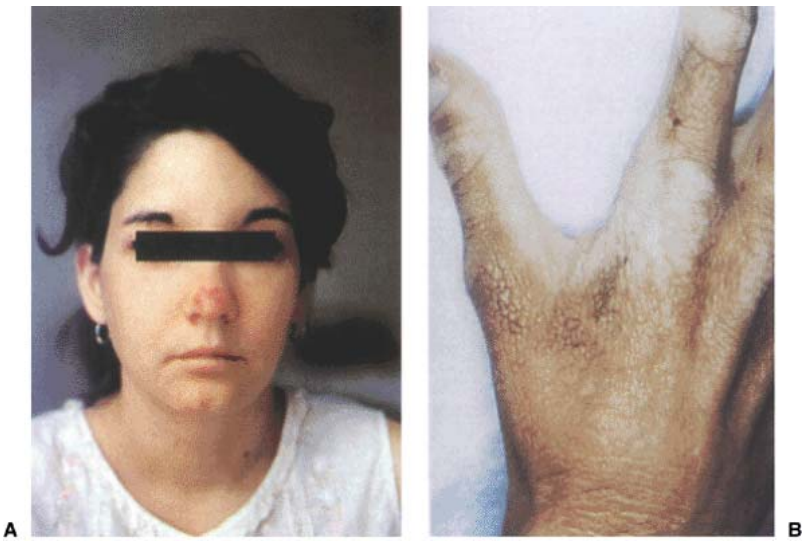
P.1104

P.1105

The activation of complement may also be a factor because complement is depleted in serum containing protoporphyrin when the serum is exposed to light (207). Mast cells release serotonin and arachidonic acid when incubated with protoporphyrin and exposed to ultraviolet light; this may contribute to the photosensitivity as well (208).



▪ **Figure 38.12** Protoporphyrin metabolism in protoporphyria. As a consequence of deficient ferrochelatase activity, protoporphyrin accumulates in heme-forming tissues—primarily the bone marrow, with a variable contribution from the liver. This excess protoporphyrin undergoes biliary excretion. Enterohepatic circulation of protoporphyrin contributes to the amount that the liver must excrete in bile. (From Bloomer JR. The liver in protoporphyria. *Hepatology* 1988;8:407, with permission.)



▪ **Figure 38.13** Cutaneous lesions in protoporphyria. **A:** Acute photosensitivity reaction showing edema of the face and erythema on the bridge of the nose following sun exposure. **B:** Chronic skin changes on the hand of a patient with protoporphyric liver disease. There is thickening and lichenification of the dorsum of the hand in areas where there was repeated sun exposure.

Hepatobiliary Disease in Erythropoietic Protoporphyria

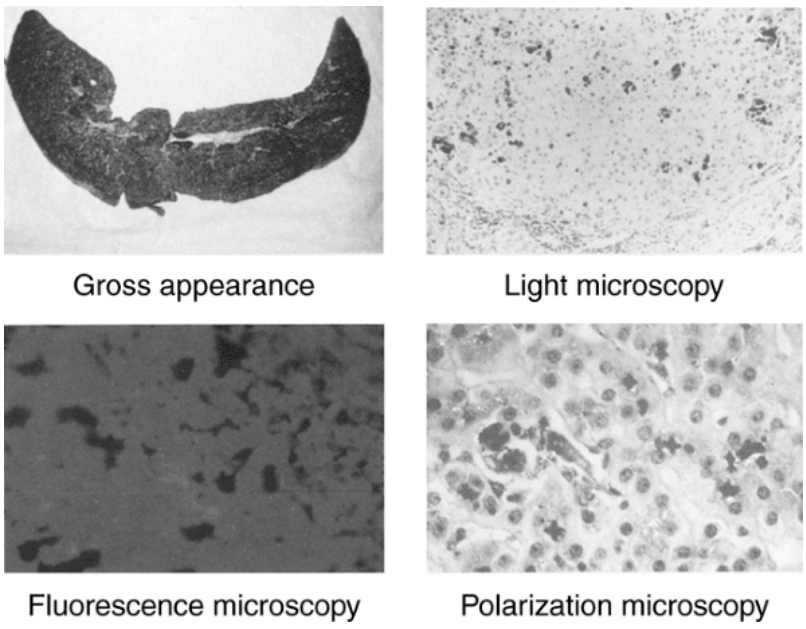
In 1963, a 6-year-old boy who had the biochemical features of EPP along with hepatosplenomegaly and abnormal liver chemistries was described. At the time of subsequent splenectomy, he was found to have a cirrhotic liver (209). In 1968, it was reported that patients with EPP may develop a liver disease that progresses to liver failure (210). The frequency remains uncertain, although less than 10% of patients appear to develop this complication.

Severe liver disease has occurred in both men and women, and in children and adults. A synergistic effect with alcohol has been reported (211), but liver disease usually develops in the absence of other causes of liver injury. Laboratory study results are nonspecific, showing variable hyperbilirubinemia with mild to moderate increases in serum transaminase and alkaline phosphatase levels. Red cell protoporphyrin levels are significantly higher than those in the usual patient with EPP, ranging from 1,404 to 36,800 µg/dL (211). Along with signs and symptoms of hepatic decompensation, patients have neurologic symptoms that include severe abdominal pain that often radiates into the back (212). Prognosis is poor once jaundice develops in a patient with liver disease due to EPP.

The livers of patients who die of hepatic failure or undergo liver transplantation are black in color (Fig. 38.14) because of massive deposits of protoporphyrin pigment in hepatocytes, macrophages, Kupffer cells, and biliary structures. The pigment deposits are birefringent when examined by polarization microscopy (213,214) because of the fact that they contain crystals (Fig. 38.10) (215).

The liver damage appears to be caused by the progressive accumulation of protoporphyrin in the liver. Regardless of the source of the excess protoporphyrin, the only means for its excretion is by hepatic clearance and secretion into bile (4). During this process, protoporphyrin is kept in solution through protein binding because it is a poorly water-soluble compound that aggregates in aqueous solution. Protoporphyrin may aggregate and form crystalline deposits within hepatocytes and small biliary radicals when concentrations are high. These deposits obstruct bile flow and damage hepatocytes. Experimental studies have demonstrated that protoporphyrin is also toxic to the liver when in solution. Perfusion of the isolated rat liver with protoporphyrin causes a reduction in bile flow (216). Histologic examination of the perfused liver shows canalicular dilatation and distortion, and membrane ATPase activity is reduced (217). Membrane dysfunction presumably occurs when the lipophilic protoporphyrin molecule intercalates into the membrane and alters the physical and chemical properties of the membrane (218,219). Studies with a mouse model of EPP also indicate that there may be formation of cytotoxic bile containing high concentrations of bile salts and protoporphyrin that cause biliary

fibrosis by damaging bile duct epithelium (220).



▪ **Figure 38.14** Liver disease in protoporphyria. Resected liver of a patient who underwent liver transplantation shows the liver to be black and fibrotic (*top left*). Light microscopy demonstrates diffuse pigment deposits in liver cells and the biliary radicles (*top right*). Fluorescence microscopy demonstrates hepatocytes to be fluorescent because of the presence of protoporphyrin, and polarization microscopy demonstrates birefringence (note the Maltese cross formation) because of protoporphyrin crystals (*bottom figures*).

Because protoporphyrin accumulation in the liver appears to be responsible for liver damage in EPP, patients with this complication presumably should have a greater abnormality in protoporphyrin metabolism than the usual patient. Indeed, red cell and plasma protoporphyrin levels are significantly higher in patients with liver disease than in the usual patient, and the distribution of protoporphyrin in red cells changes (221,222). The ratio of fecal protoporphyrin excretion to the total red cell protoporphyrin content decreases (221), and the ratio of the concentration of protoporphyrin to that of bile salts in bile increases (215).

Although the frequency of severe liver disease is not high in patients with EPP, histologic abnormalities are often found in liver biopsy specimens (223,224). These consist of focal deposits of protoporphyrin pigment, portal fibrosis, and inflammation. Changes in bile canalicular ultrastructure occur early in

the course of hepatic involvement (225). Patients also appear to have an increased frequency of gallstones, which contain protoporphyrin.

Management in Erythropoietic Protoporphyria

Most patients with EPP will require therapy only for photosensitivity. Sunscreens that protect against the wavelength of light (400 to 410 nm) that activates protoporphyrin should be used. Films can be installed on windows to provide protection against light of this wavelength. The oral administration of β-carotene in a daily dosage of 60 to 180 mg reduces photosensitivity in many patients.

For the patient in whom liver disease has developed, therapeutic approaches can be tried to diminish the production of protoporphyrin or facilitate its excretion. The oral administration of chenodeoxycholic acid has reduced protoporphyrin levels in some patients (226), as has the correction of iron deficiency (197). However, iron therapy must be used cautiously because in some patients, protoporphyrin metabolism worsens (227). Red cell transfusions and the administration of hematin have also been successful in diminishing protoporphyrin levels (222,228,229). Oral administration of cholestyramine and activated charcoal has been used to interrupt the enterohepatic circulation of protoporphyrin (230,231). A randomized placebo-controlled trial demonstrated that cysteine (500 mg orally twice a day) increased the time of symptom-free light exposure (232). Each of these approaches has a rational basis and does not pose a major risk to the patient. For the patient with early liver damage, administration of cholestyramine should be tried and liver chemistries and red cell protoporphyrin levels should be monitored during therapy.

If liver disease is advanced when the diagnosis is established, medical therapy cannot effectively reverse the situation. These patients often have a crisis, showing some of the features that occur during acute porphyria attacks. Their symptoms include severe abdominal pain that may radiate into the back, as well as mild hypertension, tachycardia, and weakness (212). Limited studies indicate that the condition can be stabilized by

a combination of plasmapheresis and hematin administration, which will allow the patient to be maintained until liver transplantation is carried out (229).

Liver Transplantation in Erythropoietic Protoporphyria

Liver transplantation has become the main option for patients with EPP in whom liver disease is advanced, and there have been several reports of success (233,234,235,236,237,238). During transplantation, patients are susceptible to unique problems because of their high protoporphyrin levels. They are at risk for photodamage to skin and abdominal tissue during exposure to fluorescent lights used in operating rooms (236,237,238); therefore, filters should be placed over the lights (238,239). Their tissues should also be protected from light as much as possible during the operation. Paralysis has occurred during the perioperative period from a severe axonal polyneuropathy, which is probably due to the neurotoxic effect of protoporphyrin (236,237,238,240). Exchange transfusion should not be carried out before transplantation because transfused red cells are sensitive to photohemolysis caused by the circulating protoporphyrin (241). However, plasmapheresis should be considered.

Unfortunately, liver transplantation does not correct the ferrochelatase defect in the bone marrow, and erythrocyte protoporphyrin levels remain high. Grafts are therefore susceptible to the toxic effects of protoporphyrin (238,242,243). The percentage of patients who developed significant damage in the new liver was 65% in a series of North American patients that was recently reported (244).

Rare Types of Porphyria

δ-Aminolevulinic Acid Dehydrase Deficiency Porphyria

Porphyria due to ALA dehydrase deficiency has been described in a few individuals (245,246). This autosomal recessive disorder results from the homozygous deficiency of the second enzyme in the heme biosynthetic pathway. Enzyme activity in affected patients is less than 3% of normal, and in obligate heterozygotes, 50% of normal. As predicted from the enzyme defect, patients excrete markedly elevated amounts of ALA in the urine. Of interest, urinary coproporphyrin and erythrocyte protoporphyrin levels are also increased, the mechanisms of which are not understood.

Children with this disorder have severe recurring porphyric attacks that respond poorly to medical therapy, including the use of intravenous glucose and hematin. As outlined in the management of the more common types of acute porphyria, patients with this disorder should avoid medications that may precipitate neurologic crises. There has been one report of successful liver transplantation, which resulted in resolution of the symptoms (247).

Congenital Erythropoietic Porphyria

Despite its rarity, congenital erythropoietic porphyria was the first type of porphyria to be described in the medical literature. In 1874, Schultz (86) reported

a patient who had dark urine and fragile skin since infancy. Fewer than 200 cases have subsequently been described. It is an autosomal recessive disorder that is usually diagnosed in infancy (248); only a few cases of adult onset have been reported (249,250). Skin lesions are indistinguishable from those in PCT. The teeth develop a reddish brown color and fluoresce when examined with ultraviolet light because of porphyrin deposition in the dentin. The bone marrow is filled with red cell precursors that fluoresce with ultraviolet irradiation. Unlike PCT, the liver shows little or no fluorescence. The disorder is associated with chronic hemolysis, and splenomegaly is common (251,252). The cause of hemolysis is not well understood but may be related to the toxic effect of porphyrin on the erythroid cells. Neurologic symptoms do not occur.

Treatment is difficult. Therapy is usually directed at hemolysis in an attempt to decrease heme turnover. Splenectomy is associated with a variable response, ranging from no effect to long-term remission of disease (253). Red cell transfusions decrease porphyrin excretion and ameliorate clinical symptoms but may cause iron overload (252). Intravenous administration of hematin has also been shown to decrease uroporphyrin excretion (250,252), but this has not been used as long-term therapy.

Hepatoerythropoietic Porphyria

Hepatoerythropoietic porphyria was first described by Gunther in 1967 and received its name in 1975 (244,254). It manifests early in infancy with discolored urine, photosensitivity, and skin fragility. Hemolytic anemia and splenomegaly may be present. As the patient reaches adulthood, the skin manifestations may diminish (255).

As the name implies, significant amounts of porphyrins are produced in both the liver and bone marrow, and normoblasts in bone marrow aspirates fluoresce. Markedly elevated levels of uroporphyrin are present in the urine. Erythrocyte protoporphyrin levels are also increased, mainly as the zinc chelate. The disorder is caused by a marked deficiency of uroporphyrinogen decarboxylase activity in heme-forming tissues (256,257,258,259). Studies of relatives of patients demonstrate mutations in both alleles of the gene coding for uroporphyrinogen decarboxylase enzyme (259,260). This contrasts with familial PCT, which is associated with heterozygous deficiency of uroporphyrinogen decarboxylase activity (257,261). Although a deficiency of uroporphyrinogen decarboxylase activity explains the increased excretion of uroporphyrin, the cause of elevated zinc protoporphyrin levels is unexplained.

A nonspecific hepatitis has been reported, and mild increases in serum transaminase levels are common. In

contrast to PCT, hepatic siderosis has not been reported and serum iron study results are normal. Fibrosis/cirrhosis is also uncommon.

There is little information available about therapy. Treatment is designed primarily to protect the skin from sunlight.

Dual Porphyrias and Harderoporphyria

Occasionally families have been described in which individuals have a deficiency of more than one enzyme of the heme biosynthetic pathway. This includes patients who have coexistent variegate porphyria and PCT (262) and those in whom deficiencies of PBG deaminase and uroporphyrinogen decarboxylase activity cause symptoms of AIP, PCT, or both (263). These conditions are called *dual porphyria*.

A French family has been described in which coproporphyrinogen oxidase activity is approximately 10% of normal in three homozygous individuals and 50% of normal in heterozygotes. Instead of increased urinary excretion of coproporphyrin, which would be expected as a result of the enzyme deficiency, the affected individuals excreted 3-carboxyl porphyrin (harderoporphyrin) in excess amounts (264). Kinetic studies indicated that the defect in the mutant enzyme caused 3-carboxyl porphyrinogen to dissociate from the enzyme more readily than normal. Harderoporphyria has also been reported in a German family (265).

Secondary Porphyrinuria

Several nonporphyric disorders are associated with an increase in the urinary excretion of porphyrins, particularly coproporphyrin, in a condition termed *secondary*

porphyrinuria (Table 38.5). Patients with these disorders may also have abdominal pain and other symptoms of acute porphyria, which can present a diagnostic dilemma. If this situation is encountered, it is important to remember that patients who are symptomatic with acute porphyric attacks have increased urinary excretion of ALA and PBG. With the exception of lead poisoning and hereditary tyrosinemia, urinary excretion of these compounds is normal in all the conditions associated with secondary porphyrinuria. The amount of urinary ALA and PBG excretion should therefore be determined to distinguish between acute porphyria and secondary porphyrinuria.

Table 38.5. Causes of Secondary Porphyrinuria

Hepatobiliary disorders	Acute and chronic hepatitis Alcoholic liver disease Cirrhosis (alcoholic and nonalcoholic) Cholestatic disorders
Toxins	Heavy metal poisoning (e.g., lead, arsenic, gold, iron) Benzene and benzene congeners Haloalkanes and haloaromatic compounds
Hematologic disorders	Aplastic, hemolytic, and pernicious anemias Leukemias Hodgkin's disease
Miscellaneous	Diabetes Hereditary conjugated bilirubin disorders (Dubin-Johnson and Rotor's syndromes) Bronze baby syndrome

Lead Poisoning

Lead poisoning is associated with several abnormalities in porphyrin metabolism because lead inhibits more than one enzyme (i.e., ALA dehydrase, coproporphyrinogen oxidase, and ferrochelatase) in the heme biosynthetic pathway. Erythrocyte levels of zinc protoporphyrin are elevated in patients with lead poisoning (193); this measurement has been used to screen for the disorder. Lead poisoning is also associated with increased urinary excretion of ALA and coproporphyrin. Because abdominal pain is a prominent clinical feature, and patients may also have peripheral neuropathies, it is possible that the mechanism for neurologic dysfunction in lead poisoning is the same as that in the acute porphyrias. Indeed, hematin has been used as adjunctive therapy in lead poisoning and causes a significant diminution in urinary ALA excretion (266).

Hereditary Tyrosinemia

Hereditary tyrosinemia type I is a metabolic disorder characterized by liver damage that develops during infancy and may progress rapidly to cirrhosis, with the subsequent development of hepatocellular carcinoma. Patients with this disorder accumulate succinylacetone (4,6-dioxoheptanoic acid) and succinylacetoacetate because of a

block in tyrosine metabolism at the level of the fumarylacetoacetase reaction (112). Succinylacetone is a potent inhibitor of ALA dehydrase (267,268) and therefore results in increased urinary excretion of ALA. Some patients also have neurologic manifestations resembling those of the acute porphyrias (269). The mechanism of neurologic dysfunction may be the same because patients with tyrosinemia respond to the intravenous administration of hematin (270). The biochemical abnormalities are corrected by liver transplantation, although minor defects, which are thought to be of renal origin, persist (271).

Miscellaneous Hepatobiliary Diseases

Because bile is a route of porphyrin excretion, any hepatobiliary disease in which bile formation is impaired may cause a diversion of porphyrins to the urine (272,273). Therefore, urinary excretion of coproporphyrin increases the most in hepatobiliary diseases. Uroporphyrin levels may also increase, but protoporphyrin is not excreted in urine, even in the face of severe cholestasis, because of its poor water solubility.

When there is biliary obstruction, urinary coproporphyrin, whose excretion is increased, contains a higher proportion of the type I isomer than usual (274). Two patterns of excretion have been observed in parenchymal liver diseases. In alcoholic cirrhosis, the increased excretion of urine coproporphyrin is associated with a proportion of coproporphyrin I in urine similar to that seen in normal individuals, whereas the pattern in other types of parenchymal disease is more like that seen in biliary obstruction. However, because of a significant overlap among these conditions, the coproporphyrin isomer distribution in urine cannot be used in the differential diagnosis of hepatobiliary disorders. Acute ingestion of alcohol may also cause a significant increase in urinary excretion of coproporphyrin, usually beginning 2 to 4 days after intoxication.

Although impaired biliary excretion is probably the main factor responsible for increased urinary porphyrin excretion in hepatobiliary disorders, there may also be an alteration in heme synthesis. Hepatic ALA synthase activity is increased in homogenates of cirrhotic livers (275), which suggests that there may be an increased rate of porphyrin production. Experimentally, it has been shown that acute ethanol administration also increases hepatic ALA synthase activity (276).

Pseudoporphyria

Pseudoporphyria is an entity with clinical and histologic features similar to those of PCT but with normal or near normal porphyrin levels (277,278,279). Pseudoporphyria is characterized by vesicles, bullae, skin fragility, milia, and scarring on the sun-exposed skin. Like PCT, the dorsal surface of the hands are most commonly affected. The histologic and immunofluorescent characteristics of pseudoporphyria are similar to those of PCT. Histologically, there are subepidermal bullae. Direct immunofluorescence reveals granular deposits of immunoglobulin G (IgG) and C3, most commonly at the dermoepidermal junction. Pseudoporphyria has been described in patients with chronic renal failure with or without dialysis, with certain medications, and with ultraviolet A radiation. The medications include nonsteroidal anti-inflammatory drugs

(highest reported incidence with naproxen), antibiotics including tetracycline, multiple sulfur-bearing diuretics, and isotretinoin (280). Ultraviolet exposure includes ultraviolet A tanning beds, psoralens plus ultraviolet A therapy, and excessive sun exposure. The exact pathogenesis is unknown but may involve photosensitivity and oxidation stress. Treatment involves discontinuation of suspected agents and sun protection. Two children with hemodialysis-associated pseudoporphyria were successfully treated with the antioxidant *N*-acetylcysteine (277).

Annotated References

Anderson KE, Bloomer JR, Bonkovsky HL, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. *Ann Int Med* 2005;142:439–450.

This paper, authored by a group of experts in the field, provides current recommendations for the diagnosis and management of the four types of porphyria in which attacks of neurovisceral symptoms occur.

Grandchamp B, Puy H, Lamoril J, et al. Review: molecular pathogenesis of hepatic acute porphyrias. *J Gastroenterol Hepatol* 1996;11:1046–1052.

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The pathologic changes in the liver in porphyria cutanea tarda are reviewed, pointing out the distinctions between those changes that appear to be specific to the porphyrias and those that may be related to ethanol abuse or iron overload.

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Chapter 39

Nonalcoholic Fatty Liver Disease

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Curtis K. Argo

Key Concepts

- Nonalcoholic fatty liver disease (NAFLD) is emerging as the most common liver disorder in industrialized countries and in many developing countries. It exists as a histologic spectrum, ranging from simple steatosis or steatosis with only mild inflammation (types 1 and 2 NAFLD) to more severe steatohepatitis (types 3 and 4 NAFLD or nonalcoholic steatohepatitis [NASH]). Types 1 and 2 NAFLD infrequently progress to cirrhosis but types 3 and 4 NAFLD (i.e., NASH) progress to cirrhosis in as many as 15% to 20% of patients. Progression is often silent, and paradoxically, it is often associated with normalization of the aminotransferases. In histologically advanced cases, the characteristic microscopic findings are lost and patients often present with "cryptogenic cirrhosis." In addition to the usual complications of cirrhosis and portal hypertension, hepatocellular carcinoma is now recognized as a late complication of NAFLD.
- Although NAFLD can occur in relatively lean individuals, obesity and type 2 diabetes remain the best characterized risk factors and are predictive (along with older age and hypertriglyceridemia) of the severity of underlying liver histology. Many lean patients with fatty liver actually have increased mesenteric fat deposits (central obesity), and ethnic variation exists in the relationship between body mass index (BMI) and visceral adiposity. In a past series of obese patients, liver biopsy results were normal in only 10%. Approximately 5% of patients have occult cirrhosis and 85% will have steatosis. In the latter, one third have NASH. The prevalence of liver disease among patients with type 2 diabetes and/or hyperlipidemia has not been as well characterized but is thought to be high. Underlying liver disease in these patients has potentially serious implications for treatment of obesity, diabetes, hypertension, and hyperlipidemia—all features of the metabolic syndrome.
- Although insulin resistance is neither absolutely essential nor sufficient for the development of steatohepatitis, the association is so strong that NAFLD, and more importantly NASH, can be considered a part of the metabolic syndrome and systemic lipotoxicity. As such, it is not surprising that lipid peroxidation is now recognized as the underlying mechanism of hepatocyte injury leading to cytokine activation and fibrosis. Steatosis results from excessive delivery of fatty acids to the liver as a result of peripheral insulin resistance, but de novo hepatic synthesis of triglycerides and impaired export of fatty acids (apolipoprotein metabolism) also contribute to the condition. Variation in the antioxidant influences the risk of subsequent cellular injury.

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- Exercise and dietary alterations are cornerstones of therapy and may result in histologic improvement. Simple steatosis probably does not warrant pharmacologic therapy but the presence of fibrosis needs a more aggressive approach. Weight loss supplements and weight-reduction surgery may be successful in some but carry a risk of complications. Other potential therapeutic modalities include the use of antioxidants and cytoprotective agents, insulin sensitizers (e.g., biguanides and thiazolidinediones [TZD]), and possibly antihyperlipidemic agents (e.g., fibric acid derivatives and 3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors), although the latter have been inadequately studied. Confounding variables (e.g., exercise and diet) and sampling error on liver biopsy samples limit interpretation of existing literature. These limitations have led to the need for incorporation of other endpoints in controlled treatment trials including anthropometric indices, measures of physical conditioning, hepatic fat content, indices of lipid peroxidation, measures of insulin signaling, and possibly systemic measures of hepatic fibrosis.

Nonalcoholic fatty liver disease (*NAFLD*), is an umbrella term that includes a range of conditions ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) (1,2). As apparent from the volume of literature published in just the last 3 years (well over 1,000 articles), it is increasingly recognized as a potentially serious condition, which can progress to cirrhosis, liver failure, and hepatocellular cancer (HCC) and has a worldwide distribution (3,4,5,6,7,8,9). The spectrum of clinical severity, in part, reflects the normal role that the liver plays in fat metabolism— a fat storage site where lipid peroxidation can lead to injury and activation of profibrotic cytokines in some individuals. The explanation for why some people develop steatosis and others do not and why some with steatosis develop injury is related to variable expression of obesity, type 2 diabetes, and the metabolic syndrome—conditions that represent a complex mixture of genetic predisposition and environmental factors (10). Several mechanisms appear to promote the accumulation of hepatic fat: De novo synthesis of triglycerides, impaired secretion of lipoprotein and, perhaps the most important in typical patients, increased delivery of fatty acids to the liver. These abnormalities correlate with central obesity and physical conditioning, providing the basis for conservative management of NAFLD with exercise, dietary changes, and weight loss. The prominent role of insulin resistance provides the basis for several of the most promising forms of pharmacologic intervention with insulin-sensitizing agents. Between these two broad categories of treatment, cytoprotective and antioxidant therapy remain under investigation as possible means of reducing oxidative injury.

Clinical and Histologic Criteria and Terminology

Although the exact histologic criteria continue to be debated, the term *NASH* is now widely regarded as the more severe form of "NAFLD." Not as widely accepted is the distinction between "primary" NASH (usually associated with obesity and diabetes without other precipitating factors) and "secondary" NASH (associated with a specific disease or some medications) (Table 39.1). This distinction is limited because shared risk factors point to a common pathogenesis of "primary" NASH and some of the conditions that have been associated with "secondary" NASH. The presence or absence of insulin resistance may be a useful distinguishing feature. As such, steatohepatitis associated with toxin exposure may qualify as a distinct entity while many other "secondary forms" may actually represent an exacerbation of "primary" NASH (See "Epidemiology" and "Other Conditions Associated with Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis—"Secondary" Nonalcoholic Steatohepatitis").

Clinical Criteria

By definition, the criteria for NASH require exclusion of alcohol as an etiology. The acceptable level of alcohol consumption is variable but the daily alcohol intake can be conservatively fixed as not exceeding 20 g/day in men and 10 g/day in women—levels below the risk level associated with increased risk of cirrhosis (30 g/day in men and 20 g/day in women) (11,12,13). However, these "cutoffs" leave an unresolved gray area in which a patient prone to NASH may consume alcohol

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Sclose to the threshold for liver injury or may have consumed more significant alcohol (measured as lifetime exposure) in the past,

leaving open the question of one chronic problem (NASH) superimposed on a past injury. These issues are further discussed in the subsequent text.

Table 39.1. Definitions and Terms

1. *NAFLD.* Indicates the presence of fatty infiltration of the liver, defined as fat exceeding 5%–10% of liver weight and frequently taken as fat in >5%–10% macrosteatotic hepatocytes in biopsy specimens. Microsteatosis is an underappreciated aspect because of limitations of routine staining techniques. The term *NAFLD* includes the term *NASH*.
2. *Simple steatosis.* A type of fatty infiltration (NAFLD) with no or minimal inflammation and no fibrosis. This is synonymous with type 1 disease, as classified by Matteoni (7) (see Table 39.2).
3. *NASH*. A type of NAFLD with inflammation, ballooned hepatocytes, and/or fibrosis, usually beginning around the central vein, which may progress to cirrhosis. This is synonymous with type 3 or 4 disease, as classified by Matteoni (7) (see Table 39.2).
4. *"Primary" NAFLD or NASH*. A term occasionally encountered in the literature but not uniformly accepted. It indicates typical NAFLD or NASH associated with central obesity and often type 2 diabetes mellitus but without a specific, additional etiology. The likelihood that many cases of "secondary" NAFLD or NASH represent unrecognized or exacerbated "primary" NAFLD or NASH makes the term less useful.
5. *"Secondary" NAFLD or NASH*. NAFLD or NASH associated with a specific problem such as a toxin. Use of the term *secondary* NAFLD or NASH implies the absence of insulin resistance. Many patients previously classified as "secondary" may have exacerbation of underlying "primary" NASH, making this distinction less useful.
6. *"Presumed" NASH or NAFLD.* Several epidemiologic and pediatric studies have utilized a presumptive diagnosis of NAFLD or NASH on the basis of abnormal liver enzyme levels, negative results of viral studies, and echogenic or "bright" liver on ultrasonography consistent with fatty infiltration. (See "Imaging in Nonalcoholic Fatty Liver Disease: Ultrasonography, Computed Tomography Scan, Magnetic Resonance Imaging and Magnetic Resonance Proton Spectroscopy").

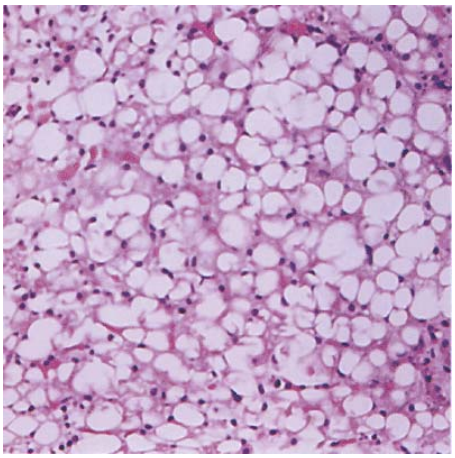
NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

Histologic Criteria

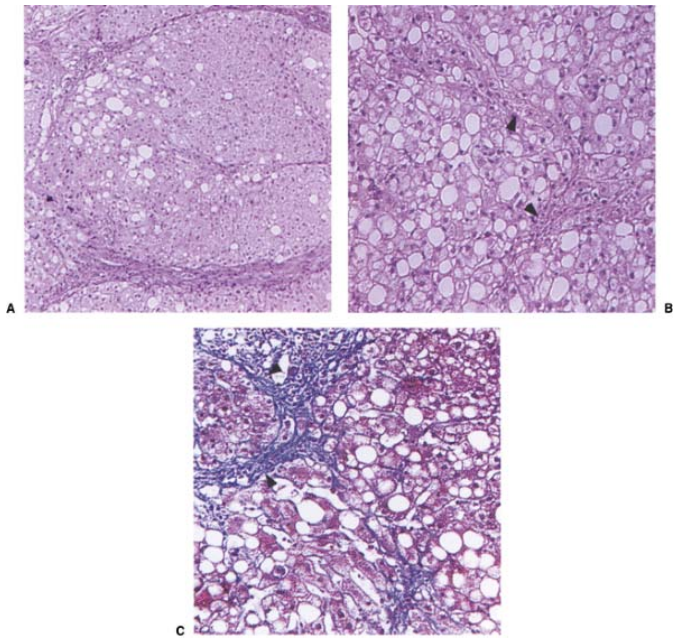
Steatosis, defined as hepatic fat exceeding 5% to 10% of total weight (14) and usually taken as fat identifiable in more than 5% to 10% of hepatocytes by light microscopy, is an essential feature of NAFLD. Within the spectrum of NAFLD, the term *NASH* indicates a more severe type of liver injury and worse prognosis compared to "simple steatosis," which is distinguished by the absence of inflammation or fibrosis and appears to have a long-term stable course (Fig. 39.1) (15). Beyond this distinction, even accomplished pathologists debate the relative importance of specific histologic variables (16). Interestingly, more or less restrictive definitions of the term *NASH* appear to influence patient demographics, suggesting the presence of gender-based variation in disease expression (17).

Important histologic variables include fibrosis (usually sinusoidal in a pericentral vein or zone 3 distribution), ballooned hepatocytes sometimes containing Mallory hyaline and lobular inflammation (Figs. 39.2 and 39.3). Glycogenated nuclei are common (18,19). Other variables include portal injury, apoptotic bodies, microvesicular steatosis (much more evident using specialized fixation techniques such as osmium), and lipogranulomas (Figs. 39.3 and 39. 4) (20,21). Substantial concordance between observers has been reported for the extent of steatosis, location and severity of fibrosis, and balloon degeneration (22). The degree of fibrosis has been organized into a staging system developed by Brunt et al. (Table 39.2) (23). In addition, although not uniformly accepted, a useful histologic classification scheme (Table 39.2) for NAFLD has been proposed and is widely referenced. It ranges from simple steatosis to the more severe steatosis with balloon degeneration and Mallory bodies or fibrosis (7). Other scoring systems have also been proposed (24) but the most significant refinements in the histologic assessment has been the development of NASH activity

index (NAI) and NASH activity score (NAS), discussed further under "Scoring of the Biopsy (Nonalcoholic Steatohepatitis Activity Index, Nonalcoholic Steatohepatitis activity score)."



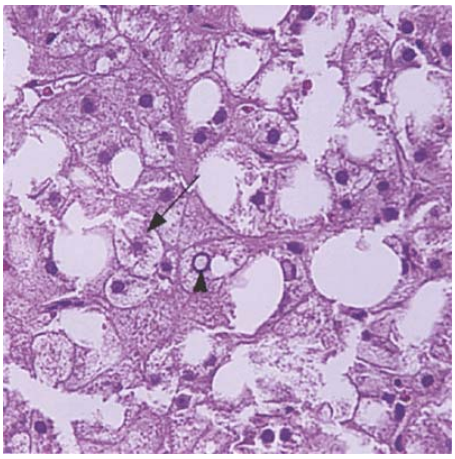
• **Figure 39.1** Simple steatosis: The patient is a 47-year-old woman with mild obesity and an idiopathic, neurodegenerative disease and hepatomegaly. The biopsy specimen showed only minimal inflammation and no fibrosis. No inciting agents were identified to explain the liver condition (hematoxylin and eosin, 200×).



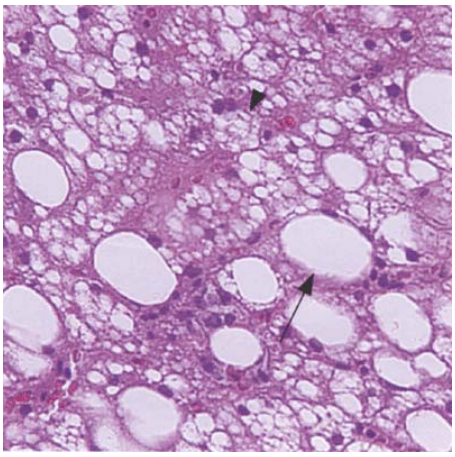
• **Figure 39.2 A:** Nonalcoholic steatohepatitis (NASH) with cirrhosis (stage 4). **B:** Early stage 3 (bridging fibrosis) with hematoxylin and eosin stain. **C:** The same biopsy accentuating the presence of bridging with a Masson trichrome stain. The specimen in **(A)** is from a 65-year-old women with moderate obesity and type 2 diabetes. She does not have complications of portal hypertension. The presence of macrovesicular steatosis, inflammation, and cirrhosis allows the diagnosis of NASH with cirrhosis (stage 4) (100×, hematoxylin and eosin). Specimens **(B)** (200×, hematoxylin and eosin) and **(C)** (200×, trichrome) are from her 40-year-old son who has mild liver enzyme abnormalities and mild (mostly truncal) obesity (body mass index = 30) without diabetes. NASH with fibrosis, mildly apparent on the hematoxylin and eosin stain **(B)**, is accentuated with trichrome staining **(C)**, which demonstrates bridging consistent with stage 3. *Arrowheads* in **(B)** and **(C)** define a fibrotic bridge bordering a regenerative nodule. These slides also illustrate a familial pattern seen in approximately 20% of patients.

"Presumed" Nonalcoholic Fatty Liver Disease

In several large epidemiologic studies, the diagnosis of "presumed NAFLD" has been made on the basis of noninvasive testing (See "Epidemiology") (12). In general, such studies have utilized abnormal transaminases in the absence of other known liver disease and/or liver ultrasonography to make the diagnosis of fatty liver disease. However, the relationship between the diagnosis of "presumed" NAFLD and the histologic activity, stage, and prognosis is unreliable. Indeed, one of the major limitations of these studies is the inability to distinguish NASH from less severe forms of fatty liver such as simple steatosis.



• **Figure 39.3** Balloon degeneration, Mallory hyaline, and glycogenated nucleus in nonalcoholic steatohepatitis. *Long arrow* indicates accumulation of perinuclear eosinophilic material (Mallory hyaline) in a ballooned hepatocyte (400×, hematoxylin and eosin). Agreement on what constitutes Mallory hyaline is sometimes hard to obtain. Ubiquitin stain, although infrequently used, can be employed to highlight Mallory hyaline (not shown). Also shown is a pale, glycogenated, nucleus (*arrowhead*), which is more typical of NASH compared to alcohol-related liver disease.



• **Figure 39.4** *Micro-* and *macrovesicular steatosis* in nonalcoholic steatohepatitis. *Arrowhead* indicates a cell with small droplets of fat in addition to the more apparent and typically large droplet in macrovesicular steatosis (*long arrow*, 400x, hematoxylin and eosin). Special stains or fixation techniques, such as osmium tetroxide fixation (not shown), can be used to accentuate the often overlooked microvesicular component.

Cryptogenic Cirrhosis

Serial biopsy studies have established the potential progression of NASH to a stage of "bland" cirrhosis with loss of characteristic histology (Fig. 39.5) (25,26). The loss of fatty infiltration may be due to altered blood flow or decreased sinusoidal permeability and impaired lipoprotein delivery as the liver becomes fibrotic (27,28,29). A number of additional studies have strongly suggested that many cases of "cryptogenic" cirrhosis, a remarkably homogenous group (Fig. 39.6), are the result of such a process (30,31,32,33,34,35,36,37,38). Approximately two thirds of patients with this diagnosis, among the most common indications for liver transplantation, have major risk factors for NAFLD (e.g., obesity and diabetes). In a series of patients undergoing transplantation for "cryptogenic" cirrhosis, definitive features of NASH were evident in 17 of 30 and minor features were seen in an additional 10 patients (39). The significantly increased frequency of steatosis and steatohepatitis after transplantation for cryptogenic cirrhosis further support this relationship (40). On the basis of these associations and predominant histologic findings (and recognizing that other conditions are also involved with cryptogenic cirrhosis), a classification system of cryptogenic cirrhosis can be formulated, as shown in Table 39.3 (41).

Table 39.2. Classification and Stages of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis

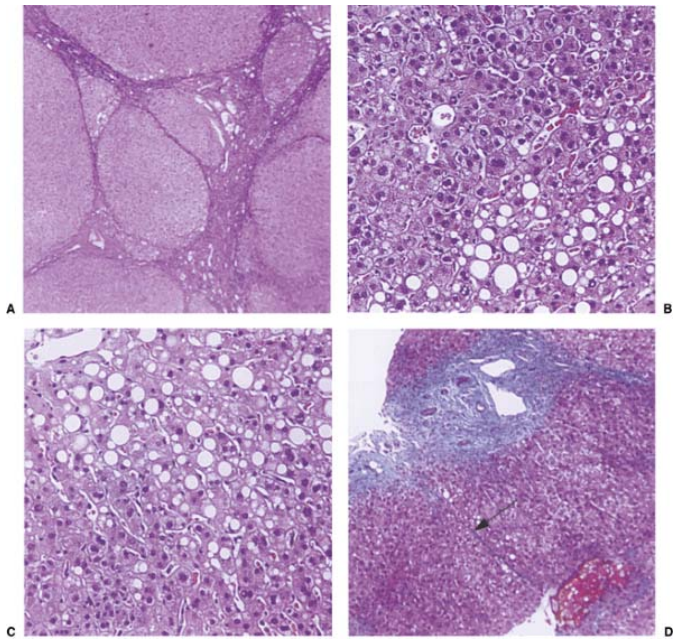
Fibrosis *Stages* of NASH (Brunt et al. (23))
Stage 1: Zone 3, pericentral vein, sinusoidal or pericellular fibrosis
Stage 2: Zone 3 sinusoidal fibrosis and zone 1 periportal fibrosis
Stage 3: Bridging between zone 3 and zone 1
Stage 4: Regenerating nodules, indicating cirrhosis
***Types* of NAFLD (Matteoni et al. (7))**
Type 1: Simple steatosis (no inflammation or fibrosis)
Type 2: Steatosis with lobular inflammation but absent fibrosis or balloon cells
Type 3: Steatosis, inflammation, and fibrosis of varying degrees (NASH)
Type 4: Steatosis, inflammation, ballooned cells, and Mallory hyaline or fibrosis (NASH)

NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease.

Focal Steatosis and Focal Sparing

In a series of patients with various forms of fatty liver disease detected radiographically, focal steatosis was evident in approximately 15% and focal sparing (usually of the caudate lobe) was seen in 9% (42). Variation in blood flow (with resulting differences in

insulin exposure and nutrient delivery) is thought to explain both focal-sparing and focal steatosis (43,44). A relationship to insulin exposure has long been suspected as a factor because of the development of focal steatosis in continuous ambulatory peritoneal dialysis (CAPD) patients exposed to insulin in the peritoneal dialysis fluid (45,46). Histologically, the lesions vary from simple steatosis to steatohepatitis (45). Cirrhotic nodules have also been shown to occasionally have focal fatty change, possibly unrelated to fatty liver disease (47,48).



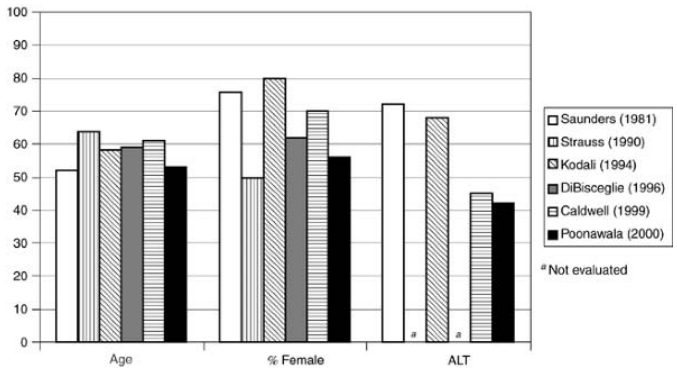
• **Figure 39.5** Development of nonalcoholic fatty liver disease (NASH) after transplantation for cryptogenic cirrhosis. **A:** Explanted liver from an obese, diabetic man showing "bland" cirrhosis (40×, hematoxylin and eosin). **B:** Two years later (200×, hematoxylin and eosin), a repeat biopsy for abnormal liver enzymes revealed steatosis that was persistent and associated with mild inflammation at 3 years **(C)** (200×, hematoxylin and eosin). **D:** Four years after transplantation the patient developed ascites and repeat biopsy showed early cirrhosis with bridging and diminished fatty infiltration (100×, hematoxylin and eosin). *Long arrow* demonstrates a fibrous band.

Historic Perspective

Early Observations

A long-recognized association between the liver and fat storage is mirrored in a popular explanation of the origin of the Latin term for liver, *ficatum*, and the corresponding modern Greek term, *sycoti*, both of which were derived from the common name for fattened animal livers, *iecur ficatum* and *hepar sykoton*, respectively (D. Tniakou, personal,

2005). A more scientific appreciation of fatty liver emerged in the 19th century when Virchow classified various types of fatty infiltration of the liver (49). The color, shape, and firmness of a fatty liver were described and fat-globules were proven to be within hepatic cells (50). Morgan, in the 1870s, described an association with obesity and overeating (51). This was extended many years later by Zelman, who reported the existence of liver damage with fibrosis and early cirrhosis in obese patients without a significant history of alcohol consumption (52). More recently, this concept resurfaced in patients who had undergone bypass surgery for morbid obesity (53,54,55,56,57,58). This was widely attributed at the time to postoperative, protein-calorie malnutrition or intestinal bacterial overgrowth, although a correlation between obesity and diabetes and potential liver damage was not strongly emphasized.



• **Figure 39.6** Past series of patients with cryptogenic cirrhosis. Previous case series have shown a female predominance, onset in sixth or seventh decade, and mildly abnormal alanine transaminase (ALT) levels. [Series from references (30,31,32,33,34,35).]

Table 39.3. Cryptogenic Cirrhosis—Proposed Classification

Class 1—cirrhosis with features of steatohepatitis including scattered steatosis, ballooned hepatocytes, possibly with Mallory

bodies, and glycogenated nuclei
Class 2—cirrhosis with features of autoimmune disease including plasma cells or granulomas
Class 3—cirrhosis with features of biliary obstruction including proliferation of bile ducts and cholestasis
Class 4—bland cirrhosis: Cirrhosis lacking other distinguishing features

From Contos MJ, Cales W, Sterling RK, et al. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. *Liver Transpl* 2001;7:363–373 and Ayata G, Gordon FD, Lewis WD, et al. Cryptogenic cirrhosis: clinicopathologic findings at and after liver transplantation. *Hum Pathol* 2002;33:1098–1104.

The Ethanol Conundrum

As previously noted, the cutoff levels for classification of "nonalcoholic" versus "alcoholic" steatohepatitis (ASH) remain unresolved. Clearly, there are many patients with NASH and related cirrhosis without a history of current or past ethanol exposure ("teetotalers"). Before this recognition, a common experience was nicely summarized by Ludwig in his original description of NASH:

> *"…we have encountered patients who did not drink, who had not been subject to bypass surgery, and who had not taken drugs that may produce steatohepatitis, yet had in their liver biopsy specimens changes that were thought to be characteristic of alcoholic liver disease. In these instances, the biopsy evidence sometimes caused clinicians to persevere unduly in their attempts to wrench from the patient an admission of excessive alcohol or to obtain a confirmation of such habits from relatives of the patients. Thus, the misinterpretation of the biopsy in this poorly understood and hitherto unnamed condition caused embarrassment to the patient and physician."*(2)

Nonetheless, it is widely suspected and recently documented that approximately 10% of patients classified as having NASH actually have had a significant lifetime exposure to ethanol when a more structured

history is obtained (59). Intuitively, synergy between ASH and NASH seems likely by the association of more severe alcohol-related liver disease with obesity (60,61,62,63). However, one study has indicated a lower risk of severe steatohepatitis among obese patients consuming moderate alcohol, possibly mediated by effects on insulin signaling (64,65). Because of the widely perceived health benefits of moderate ethanol ingestion (e.g., red wine), the difficulty in sorting out NASH from alcohol-related liver injury is likely to persist. Immunohistochemical stains for insulin receptors and regulators may provide a means of distinguishing the prominence of one pathway over another but they have not been validated and clinical utility not yet confirmed (66). With the possible exception of glycogenated nuclei (increased in NASH), other histologic features do not reliably distinguish between ASH and NASH (18,67).

A number of laboratory tests have been proposed to make this distinction including carbohydrate-deficient transferrin; however, none has proved satisfactory (68). Transaminase ratios may provide guidance: The aspartate transaminase (AST) to alanine transaminase (ALT) ratio is typically less than 1 in early or mild NASH, between 1 and 2 in more severe NASH, and more than 2 in more severe ASH (See "Clinical and Laboratory Findings"). The extent of elevation of γ-glutamyl transpeptidase (GGT) levels may also be useful (seldom >400 in NASH), but none of these relationships is entirely reliable and may be obscured by concomitant medication use. For these reasons, the history remains the most commonly used means of assessing alcohol consumption, especially when repeated by multiple health care providers over time.

"Hepatogenous" Diabetes

Complicating the relationships between insulin resistance, hyperinsulinemia, and NAFLD is the relationship between liver disease and diabetes, which has long been referred to as *hepatogenous diabetes* (a term coined by Naunyn in the early 1900s) (69). The issue is whether hyperinsulinemia coincides with the development of liver disease, as appears to be the case in NAFLD, or follows the development of liver disease, as in hepatogenous diabetes. A number of papers have established an increased prevalence of diabetes in cirrhosis of various etiologies (70). Impaired insulin sensitivity rather than decreased insulin metabolism from portosystemic shunting, are postulated to explain this condition (71,72). The role of cirrhosis-related abnormal skeletal muscle metabolism, a major target of insulin and one of earliest abnormalities detectable in type 2 diabetes, is yet to be investigated (73).

Epidemiology and Prevalence in High-Risk Groups

NAFLD is one of the most common of all liver disorders, especially in industrialized countries, and represents a significant source of disease (74,75,76,77,78,79,80). The estimated prevalence in the general population ranges from 2.8% to 20%, depending on the criteria used for estimation (81). In a series of 150 consecutive patients with abnormal liver enzymes for at least 6 months, 40% had steatosis, 15% had hepatitis C, and 2% had nonalcoholic steatohepatitis (82). In another series of 81 patients with abnormal liver enzymes and a negative serologic workup, 50% of the patients had steatosis and 32% had steatohepatitis (83). In the primary care setting, NAFLD accounts for approximately one third of cases of suspected chronic liver disease cases (84). Obesity, type 2 diabetes and hyperlipidemia have been the most constant conditions associated with steatosis and steatohepatitis and are predictors of more severe histologic disease (85). In a large study of risk factors for the presence of NAFLD at autopsy, Wanless and Lentz found mild to severe steatosis in approximately 70% of obese patients compared to 35% of lean patients and steatohepatitis in 18.5% of obese patients compared to just 2.7% of lean patients (86). Diabetes has also been identified as an independent risk factor for NASH. Bellentani et al. identified a 4.6-fold increased risk of fatty liver in obese patients compared to nonobese patients and also identified hypertriglyceridemia as a significant predictor of steatosis on liver ultrasonography (12). An overview of major conditions associated with NAFLD and NASH is shown in Table 39.4.

Obesity

Biopsy studies in obese patients (body mass index [BMI] usually >30) showed steatosis in 85%, mild to moderate fibrosis in approximately 25% to 30%, and cirrhosis in 1% to 2% (87,88). Ratzui et al. found that approximately 30% of consecutive obese patients with abnormal liver enzymes had at least septal fibrosis and 10% had cirrhosis (89). In another series of obese patients undergoing gastroplasty, Garcia-Monzon et al. observed NASH in 69%, while 22% had simple steatosis and only 8% had a normal biopsy (90). Similar to other studies, one half of those with mild or severe steatohepatitis had normal liver enzymes (91). In another group of patients undergoing bariatric surgery, Dixon et al. reported that only 4% had normal results of biopsies; 71% had simple steatosis and 26% had steatohepatitis with variable degrees of fibrosis (64). Similar results were noted in a more recent study. A compilation of histology in these series of obese patients is shown in Figure 39.7 (92).

Table 39.4. Conditions Associated with Nonalcoholic Fatty Liver

Metabolic factors
- Obesity (especially truncal or central obesity)
- Type 2 diabetes mellitus
- Hyperlipidemia (especially hypertriglyceridemia)
- Systemic lipotoxicity

Specific conditions associated with fatty infiltration of the liver
- Metabolic syndrome (hyperinsulinemia, hypertension, obesity, polycystic ovary disease)
- Lipodystrophy
- Mitochondrial diseases
- Weber-Christian disease

Bariatric (weight loss) surgery
- Jejunoileal bypass (no longer performed)
- Gastric bypass or gastroplasty (less frequent compared to jejunoileal bypass)

Medications
- Methotrexate
- Amiodarone
- Tamoxifen
- Nucleoside analogs

Parenteral nutrition and malnutrition
- Total parenteral nutrition
- Kwashiorkor
- Celiac disease

Miscellaneous
- Wilson disease
- Toxins (CCl_4, perchloroethylene, phosphorous, ethyl bromide, petrochemicals)

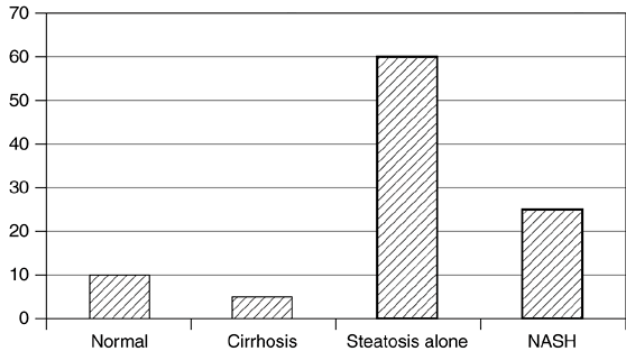
Type 2 Diabetes Mellitus

Insulin resistance is common in patients with NASH, and hyperinsulinemia plays a pathogenic role in the progression of NASH even in the absence of overt diabetes (93,94). It is estimated that up to 75% of patients with type 2 diabetes have fatty infiltration (93,95,96), although ethnicity appears to influence the prevalence significantly (see subsequent text) (97). Fatty infiltration has been noted to commonly precede the development of overt diabetes in earlier studies (98). The progression to more overt diabetes in these patients depends on peripheral fat and skeletal muscle metabolism and pancreatic islet cell vitality. The severity of liver injury worsens with the degree of abnormal glucose metabolism among obese patients (99) and the impact on the overall clinical course is significant—the standardized mortality ratio in patients with type 2 diabetes is actually higher for cirrhosis than for cardiovascular disease (100,101). Younossi et al. further demonstrated that the coexistence of diabetes in patients with NAFLD more than doubled the prevalence of cirrhosis on diagnostic biopsy from 10% to 25% (102).

Hyperlipidemia

As with type 2 diabetes, large studies revealing the true prevalence of NAFLD and associated histology within different forms of hyperlipidemia are lacking. In one study using noninvasive imaging, it was shown that two thirds of patients with hypertriglyceridemia and one third of those with hypercholesterolemia have fatty liver (103). This is probably an underestimation because significant hyperlipidemia (i.e., triglyceride, total cholesterol, or high-density lipoprotein [HDL] cholesterol) was reported in 96% of patients with NASH in one large, well-characterized series (104), although a lower range (3% to 92%) was noted in a compilation of 13 series summarized by McCullough (105). It is likely that ethnic, presumably genetic, factors will influence the true prevalence (see "Genetic Variation"). However, the close relationship between hyperlipidemia and fatty liver disease raises further practical concerns about the effects of antihyperlipidemic medications. Although acute hepatitis appears to be rare

among patients with suspected NAFLD treated with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (See "Treatment of Nonalcoholic Fatty Liver Disease"), the long-term effects have not been adequately explored (106,107,108,109).



• **Figure 39.7** Liver histology in series of obese patients. Only 10% of obese subjects have normal histology while 5% have previously unidentified cirrhosis. Approximately 85% have steatosis and one third of these have, in addition, nonalcoholic steatohepatitis (NASH) with varying degrees of fibrosis. (Adapted from findings in references 64,87,88,89,90,91,92.)

Metabolic Syndrome

Unexplained elevation of liver enzyme levels, attributed largely to NAFLD, are seen in approximately 7% of individuals meeting the criteria for the metabolic syndrome, defined by the Adult Treatment Panel-III criteria (Table 39.5) (110,111). However, this is probably an underestimation of the true prevalence, given the high prevalence of NAFLD in the general population, the high prevalence of features of the metabolic syndrome among patients with NAFLD, and the frequency of normal liver enzymes even in the setting of significant

histologic disease (the last factor obscures the true prevalence if NAFLD is detected depending on abnormal aminotransferases) (112). It has been suggested that NAFLD can cause metabolic syndrome. However, current data indicate that both hepatic steatosis and central adiposity are independent risk factors for metabolic syndrome and all three conditions appear to be united by variable degrees of insulin resistance and systemic lipotoxicity (see subsequent text) (113,114,115).

Normal Body Mass Index

NAFLD has been well documented in patients with normal BMI (5,17,116). This group appears to contain relatively younger males with milder histology, visceral or central adiposity (without overt obesity by BMI), and hyperinsulinemia. Such individuals, possibly more common in Asian populations where visceral adiposity is seen with lower BMI, are thought to represent an initial stage of the insulin resistance syndrome (117,118,119,120). These findings are consistent with the major role of body fat distribution as opposed to simply the amount of body fat in the development of insulin resistance and NAFLD (121,122,123,124).

Table 39.5. Adult Treatment Panel-III Criteria for the Metabolic Syndrome (110)

Risk factor	Definition
Abdominal obesity	Waist circumference
Men	≥102 cm (≥40 in.)
Women	≥88 cm (≥35 in.)
Triglycerides	≥150 mg/dL
High-density lipoprotein cholesterol	
Men	<40 mg/dL
Women	<50 mg/dL
Blood pressure	≥130/85 mm Hg
Blood glucose	≥110 mg/dL

Pediatric Patients

In parallel to the increasing problems of obesity, a number of papers have now established NAFLD and NASH as potentially serious problems in children (125,126,127,128,129). As with adult fatty liver disease, the problem appears to exist worldwide. The histologic severity appears to vary substantially but fibrosis and cirrhosis have been described in 18 of 24 patients in one Canadian series (130). Recently acquired rather than long-standing obesity increases the risk of fatty infiltration in this group. Ethnic variation, male preponderance, and prominent portal tract injury standout in pediatric NASH (126,131). Ethnic influences appear similar to those in the adult disease (see subsequent text), with a greater risk among children, especially boys, of Hispanic or northern European descent compared to African American pediatric patients (132).

Nonalcoholic Steatohepatitis as a Factor in Other Liver Diseases

NAFLD is so common that it can be expected to coexist with virtually any other liver disease and to possibly influence the course of that disease (133). Several studies have indicated that steatosis (possibly mediated by core protein metabolism) (134) is associated with hepatitis C (especially genotype 3) and accelerates liver disease progression (135). The association with insulin resistance and type 2 diabetes has been sufficiently strong to dub hepatitis C as a metabolic disease (136), and a measure of insulin resistance in hepatitis C has been shown to predict fibrosis (137). Although viral metabolism may be important in this association (138), many patients with hepatitis C virus and features of NASH have coexisting risk factors for metabolic syndrome and, therefore, for NASH, suggesting a synergistic effect rather than a causal relationship (139,140). Iron loading of the liver has also been suggested as a factor in the progression of NASH, although this is controversial (141,142). As recently pointed out by Powell et al. hyperferritinemia in NAFLD correlates more closely with insulin resistance than with iron overload or *HFE* gene mutations and can be reversed with weight loss (143).

Genetic Factors in Nonalcoholic Fatty Liver Disease

Ethnic Variation

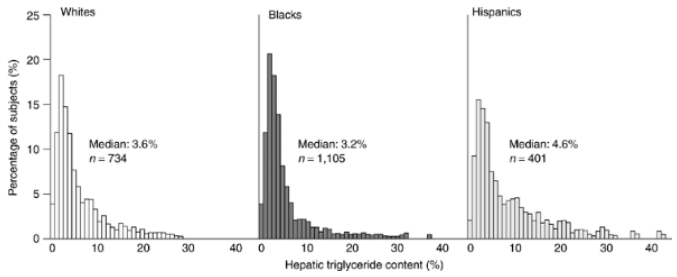
Several reports have described significant ethnic variation in the prevalence of NAFLD/NASH (144,145,146,147). This is explained, in part, by ethnic differences in the

distribution of body fat because central adiposity correlates better with fatty infiltration of the liver (and to insulin sensitivity) than does total body fat (148,149,150,151,152,153). The observation is consistent with the genetic associations of obesity and diabetes and the ethnic variation described in lipoprotein metabolism (154,155,156,157,158). Similar ethnic variation has been described in cryptogenic cirrhosis (144,159). Most of these studies, including the large epidemiologic study by Weston et al. have indicated that people primarily of African American descent seem to have a lower than expected prevalence of steatosis relative to the rates of obesity and diabetes (160). The most convincing data comes from Browning et al. who studied 2,287 subjects with magnetic resonance spectroscopy (MRS) (see subsequent text) to measure liver triglyceride content (161). The authors again noted that African Americans tended to have significantly less steatosis than Hispanic or non-Hispanic whites independent of the presence of obesity or diabetes, suggesting that additional factors influence the development of steatosis (Fig. 39.8). Similar variation in the prevalence of NAFLD among patients with diabetes has also recently been described. (Fig. 39.9).

Familial Factors

A high prevalence of afflicted first-degree relatives has been described in at least two studies of NASH (162,163). Both these studies reported an association with obesity and diabetes, and one showed a relationship with cryptogenic cirrhosis within kindreds, perhaps

explaining prior reports of familial cirrhosis (164). However, it remains unclear whether these associations represent genetic mechanisms, common environmental exposures, and shared risks or perhaps some combination of both genetic risk and common health habits. The demonstration of impaired skeletal muscle mitochondrial metabolism associated with insulin resistance in the offspring of patients with type 2 diabetes further suggests a genetic risk closely related to handling and disposition of intracellular fat (165).

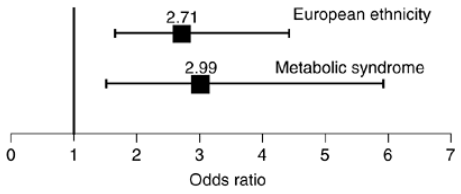


• **Figure 39.8** Distribution of hepatic triglyceride content by ethnicity as assessed by ^{1}H-magnetic resonance spectroscopy. Browning et al. (162) showed that the distribution of hepatic triglycerides content was skewed toward lower levels in blacks and slightly higher levels in Hispanics. (Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.)

Genetic Variation

The search for genetic factors has involved seeking specific polymorphisms of candidate genes involved in fat metabolism, oxidative stress and cytokine activity, and genome-wide comparative surveys of single nucleotide polymorphisms (SNPs) (166,167). Experimentally, variation in hepatic lipogenic gene expression was seen in mouse strains in which, paradoxically, greater hepatic steatosis was associated with less insulin resistance (168). In humans, microarray analysis has shown that patients with NASH underexpress genes

associated with the mitochondrial antioxidant system and overexpress acute-phase reactants that may play a role in insulin resistance (169,170). Variation in adiponectin and hormone-sensitive lipase polymorphisms (important in flux of fatty acids between the liver and periphery) has been described, as has variation in SNPs of microsomal triglyceride transfer protein (171,172). Other potential factors include the CD36 protein (e.g., fatty acid translocase—a scavenger-receptor expressed in adipose tissue where it functions as a transporter of fatty acids and oxidized low-density lipoprotein), lamin A mutations (associated with lipodystrophy), mitochondrial mutations (see subsequent text), iron overload, cytochrome P-450, peroxisome metabolism, and variation in the function of antioxidants (e.g., glutathione, vitamin E and superoxide dismutase) that are known to be depleted in conditions associated with hepatic lipid peroxidation (173,174,175,176,177,178,179,180,181,182). As previously described in patients with hepatitis C infection, a synergistic association between high expression of angiotensinogen and of transforming growth factor-β1 genotypes has also been described in obese patients with more severe NASH (183,184).



• **Figure 39.9** Diabetes, ethnicity, and nonalcoholic fatty liver disease (NAFLD). Sundaram et al. demonstrated significantly increased odds ratios for NAFLD in patients with diabetes who were of predominantly European descent compared to patients of African American descent and in patients with metabolic syndrome (97).

Clinical and Laboratory Findings

Symptoms and Signs

Specific symptoms in NASH are infrequent. Associated signs and symptoms and laboratory findings are summarized in Table 39.6. In a compilation of several studies, Reid noted an absence of specific symptoms in 48% to 100% of patients (8). However, in another study, Sanyal noted fatigue in 45 of 62 patients (73%) and right upper quadrant pain in 30 of 62 (48%) (185). This discomfort is often mistaken for gallstone disease that can also be associated with obesity and hyperinsulinemia (186). Persistence of pain after cholecystectomy or an abnormal-appearing liver at the time of surgery often precipitates a referral to the liver clinic. Hepatomegaly is usually due to steatosis but may be due to hepatic glycogenosis in patients with diabetes (187). Acanthosis nigricans has been noted in some children with NASH. The presence of palmar erythema or spider angiomas suggests cirrhosis that may very subtle—we have seen a number of patients whose initial presentation of NASH with cirrhosis was the presence of rectal varices detected at screening colonoscopy. A family history of fatty liver, unexplained liver abnormalities, or cryptogenic cirrhosis is seen in approximately 20% to 25%. A subacute form of NASH with cirrhosis probably represents unrecognized chronic disease with unexplained sudden decompensation (188,189).

Table 39.6. Signs, Symptoms, and Laboratory Features

SYMPTOMS
Asymptomatic (48%–100%)
Fatigue (~70%)
Right upper quadrant pain (up to 50%)
Occasional neurological deficits (possibly part of systemic lipotoxicity)
SIGNS
Hepatomegaly
Acanthosis nigricans in children
Palmar erythema and spider angiomas (if cirrhosis has developed)
LABORATORY FEATURES
Elevated aspartate transaminase and alanine transaminase levels (usually <2 × normal)
Mildly elevated γ-glutamyl transpeptidase level
Mildly elevated alkaline phosphatase level
antinuclear antibody positive in ~30%
Increased immunoglobulin A
Abnormal iron indices in 20% to 60% (usually not with definite hemochromatosis)

See text references.

Not surprisingly, clinical findings associated with the metabolic syndrome, including obesity, type 2 diabetes, hyperlipidemia, hypertension, hyperuricemia, polycystic ovary syndrome (i.e., insulin resistance, diabetes, obesity, hirsutism, oligomenorrhea, or amenorrhea), and gallstone disease, are common (93,94,186,190,191,192,193,194,195,196,197). Changes in body composition due to aging and cirrhosis (both associated with loss of muscle and adipose tissue) may mask a history of prior, severe, and long-standing obesity (198,199). The relationships between the hypercoagulable component of metabolic syndrome (due to plasminogen activator inhibitor-1 increased with central obesity), systemic endothelial/vascular abnormalities, and steatohepatitis have not yet been fully explored (200,201,202).

Laboratory Features

Many patients present with only abnormal liver test results, especially ALT and/or AST often detected on routine screening or during institution of weight loss therapy, or antihypertensive, antidiabetic, or antihyperlipidemic therapy. Transaminase levels are usually less than two times the upper limit of normal; from a compilation of five earlier studies reviewed by Harrison and Neuschwander-Tetri, the average AST and ALT levels were 79 IU/L and 64 IU/L, respectively (203). AST, partly mitochondrial in origin (204), correlates imperfectly with the degree of inflammatory activity and injury as does the AST to ALT ratio, in that values less than 1 are consistent with mild disease while values greater than 1 often indicate fibrosis (205). Mild elevation of GGT and alkaline phosphatase levels may also

be present but are not as well studied. GGT was predictive of more severe histology in one study, although isolated GGT level elevation is of doubtful significance in this setting (89).

Nonalcoholic Fatty Liver Disease and Normal Aminotransferases

It is now well known that patients with significant histologic liver disease, including cirrhosis, may have normal aminotransferase levels (206,207). The lack of complete correlation between histology and aminotransferases is similar to that seen with hepatitis C—the relationship is present but imperfect. It is generally accepted that elevation of AST and ALT levels indicates hepatocyte injury or, possibly, the rate of cell turnover, which results from cell injury (208). Other explanations include the possibility that the "true normal" range in obese patients may be lower than that for lean individuals (209). Indeed, ALT levels are positively correlated with central obesity and hyperinsulinemia (leading to an ongoing effort to revise normal ranges) and negatively correlated with caffeine consumption (210,211,212). In addition, the use of a thiazolidinedione medication (troglitazone) was shown to normalize transaminase levels and improve parameters of inflammation, but all patients still met criteria for NASH on follow-up biopsy (213).

Other Laboratory Abnormalities

Abnormal sinusoidal deposition of immunoglobulin A (IgA), indistinguishable from that seen with alcohol-related liver disease, has previously been described in NASH (214). Serum IgA level elevation in alcohol-related liver disease results from neoantigen formation (215). Although not as well studied, isolated elevation of serum IgA level is seen in 25% of patients with NASH and a lower serum IgG to IgA ratio is associated with more severe fibrosis (216). Antinuclear antibody is seen in approximately 35% of patients with NASH (217,218). Abnormal iron indices, including ferritin and transferrin saturation, are seen in 20% to 60% of patients, but this is not usually associated with homozygous or heterozygous genetic hemochromatosis and the relationship to disease severity remains unsettled (219). Low platelets, often misdiagnosed previously as idiopathic thrombocytopenia, warrants an additional search for hypersplenism and cirrhosis (220).

Findings of Lipodystrophy

Fatty infiltration of the liver is a common feature of the lipodystrophies (221,222). These disorders, congenital or acquired, vary in the distribution of dystrophic fat. Common features include diabetes, hypertriglyceridemia, panniculitis, and focal or diffuse loss of subcutaneous fat. The mechanism is thought to involve failure of differentiation of preadipocytes, possibly due to lipin deficiency (223). The diagnosis depends on cross-sectional imaging of an involved area to demonstrate fat atrophy. Histologic NASH was observed in eight of ten patients in a recent small series that also demonstrated improvement with leptin replacement, supporting a role for leptin deficiency (224). Cirrhosis has been described among women with the acquired variety (225,226). A familial form of partial lipodystrophy has also been described in association with NAFLD (227). On the basis of the subtle presentation of lipodystrophy and lack of easily available tests, it seems likely that there is more overlap between NAFLD and lipodystrophy than is commonly appreciated. A potentially important distinguishing feature of the more severe forms of lipodystrophy is the characteristically low leptin level (228). Lipodystrophy related to antiretroviral therapy, perhaps now the most common form of this disorder, is discussed further in subsequent text.

Findings Suggestive of Mitochondrial Disease

The systemic nature of NAFLD and the presence of morphologic mitochondrial abnormalities in liver biopsy specimens (94,229) suggest that other mitochondria-related clinical manifestations may be prevalent in NAFLD (230). Features of systemic mitochondrial disease can be observed in patients with NASH including depression, ophthalmoplegia, neurodegenerative diseases, deafness, lipomatosis, and gut dysmotility (231). Few of these have been systematically investigated, although Al-Osaimi et al. recently reported an increased prevalence of a subtle form of gaze palsy in patients with NASH (232). Insulin resistance and dyslipidemia are features of symmetric lipomatosis or Madelung's disease (associated with mitochondrial deoxyribonucleic acid [DNA] mutations) (233,234) and of maternally inherited diabetes and deafness (MIDD) syndrome (also associated with mitochondrial mutation) (235). Similar hepatic mitochondrial

DNA mutations have been identified in some patients with NASH (236). In addition, cryptogenic cirrhosis has been described in certain mitochondrialopathies (237). Underlying these seemingly unrelated problems, mitochondrial heteroplasmy could play a role in the variable expression of a mitochondrial problem in different organs (238,239).

Weber-Christian Disease

Nodular panniculitis, especially over the lower extremities, is the most distinguishing feature of classical Weber-Christian disease (240,241). Steatohepatitis is a common finding, suggesting a systemic disorder of

fat metabolism. Liver chemistries may be only mildly increased or there may be an acute steatohepatitis with jaundice and Mallory bodies may be evident. The diagnosis can be established by demonstration of fat necrosis. In distinct contrast to many other forms of NAFLD, immunosuppression has been reported to be effective therapy, suggesting an autoimmune component (242).

Imaging in Nonalcoholic Fatty Liver Disease: Ultrasonography, Computed Tomography Scan, Magnetic Resonance Imaging, and Magnetic Resonance Proton Spectroscopy

Liver imaging plays an important role as an initial diagnostic tool, in epidemiologic studies, and in the evaluation of partial liver donors (Fig. 39.10) (243). Of particular relevance to epidemiologic studies, imaging is used to assess the distribution of body fat in comparison to anthropometric measurements (244,245). For example, computed tomographic (CT) measurement of abdominal visceral fat at the L4-5 intervertebral space, adjusted for age and sex (246), is the most established technique (247). Magnetic resonance imaging (MRI) (248,249) and ultrasonography (250) have also been utilized to quantitate body fat, especially central adiposity. However, none of the conventional imaging techniques can accurately grade or stage NASH (with the possible exception of advanced MRI), and the accuracy of even detecting steatosis is limited above levels of 20% to 30% (251,252).

Ultrasonography and Elasticity

Ultrasonography can detect the presence of hepatic steatosis through increased echogenicity and sound attenuation with defined criteria for fatty infiltration (253,254,255). However, its utility is limited because of difficulty in differentiating fibrosis from fatty infiltration (256,257), misinterpretation of focal fatty sparing as a hypoechoic mass (258), and poor detection if the degree of steatosis is less than 20% to 30%. Nonetheless, as initial testing in a suspected case and for large population screening, it is a reliable and economical means of assessment. Newer techniques including measurement of hepatic elasticity (decreased with fibrosis) are promising but have not been adequately evaluated in NAFLD in which fatty infiltration may present a problem by spuriously decreasing elasticity.

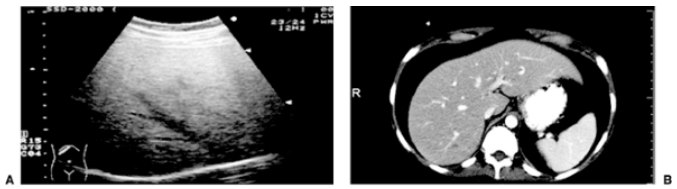
Computed Tomography

With CT, unenhanced scans remain the optimal technique to image hepatic fat when the diagnosis relies on attenuation differences between the liver and spleen (151,259,260). The sensitivity and specificity of detecting fatty liver (with spleen-minus-liver attenuation of 10 Hounsfield units) were 0.84 and 0.99, respectively, in one study (261). Recently, therapeutic trials have used the "liver-to-spleen ratio" (in Hounsfield units) in which values less than 1 are consistent with relatively greater steatosis. Contrast-enhanced CT scan has more limited utility because the optimal liver-minus-spleen attenuation differences are significantly influenced by the contrast injection rate and timing of measurement (262).

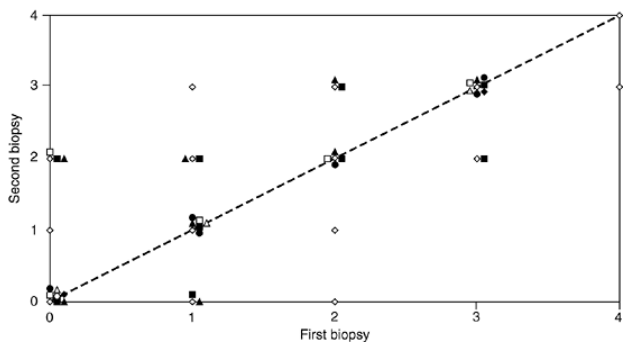
Magnetic Resonance Imaging and Spectroscopy

Conventional spin-echo MRI is insensitive in detecting fatty infiltration (263). This limitation is improved with refinements of the technique, including modified spin-echo and in-phase, out-of phase imaging (264,265). Proton (^{1}H)-MRS is another means of very accurately assessing the degree of hepatic steatosis (266,267). In one study, the correlation between fat concentration

measured in the liver biopsies and ^{1}H-spectroscopy was 0.9 ($P < 0.001$) (268). ^{31}P spectroscopy (Fig. 39.11) offers the futuristic prospect of measuring cellular chemistry and metabolic parameters including adenosine triphosphate (ATP) homeostasis in the liver, lipid peroxidation, and phospholipid content of the liver (270,271,272,273,274,275).



• **Figure 39.10** Radiologic imaging of nonalcoholic fatty liver disease (NAFLD). **A:** Sonographic appearance of NAFLD. **B:** Computed tomography (CT) appearance of NAFLD. **(A)** demonstrates a heterogeneous-appearing echotexture with findings of "bright liver" in the parenchyma; **(B)** shows a relatively hypodense liver compared to the spleen (liver-to-spleen ratio <1).



• **Figure 39.11** Biopsy sampling error. Ratziu et al. (269) show evidence of the sampling error that can occur if inadequate liver biopsy specimens are obtained. The biopsy specimens of 15 of 51 patients showed at least a one-stage difference in fibrosis stage, and 2 showed a two-stage difference from the same right flank site with slightly different needle orientation, indicating the need for samples of at least 2 to 3 cm in length using a standard 15–gauge width needle to minimize sampling error. The symbols represent the results of an individual patient's consecutive biopsies.

The Use of Liver Biopsy in Nonalcoholic Fatty Liver Disease

Liver biopsy remains the gold standard for confirming the diagnosis, staging the extent of injury, and grading the degree of activity. However, biopsy is often deferred, and a conservative course of exercise and diet is prescribed as initial steps unless the clinical evaluation indicates more advanced disease or when there is a question of medication-induced injury. Practically speaking, the importance of the biopsy is increasing as therapeutic interventions improve and with the recognition of occult cirrhosis as a significant consideration in the management of older patients with diabetes (see "Treatment of Nonalcoholic Fatty Liver Disease"). The use of surrogate tests such as serologic fibrosis markers, refined imaging techniques, or noninvasive measures of elasticity is promising but remains to be fully explored (276,277,278).

Predictors of Underlying Histology

Clinical predictors of findings on the initial biopsy specimen have been studied extensively (Table 39.7), although such noninvasive predictors should be regarded cautiously because of the high number of exceptions. Age (>40 to 50 years), the degree of obesity, the degree of diabetes or insulin resistance, hypertriglyceridemia, hypertension, family history of NASH or cryptogenic cirrhosis, complete abstinence from ethanol, transaminase level (AST and ALT) elevation (relatively weak marker), and an AST to ALT ratio more than 1 are often, but not invariably, predictive of more advanced histology, but conflicting studies exist (5,7,30,60,64,76,85,89,90,91,205,279,280,281,282,283,284,285). Other factors noted as predictive of more severe histology on the initial biopsy specimen include circulating antibodies to lipid peroxides (286) and the number of parameters positive from the Adult Treatment Panel-III criteria for metabolic syndrome (287). A number of these variables have been combined into composite scores, but these also have limited sensitivity and specificity and have not gained wide clinical acceptance. Incorporation of markers of collagen metabolism, such as serum hyaluronic acid, into a clinical score enhances the predictive value but still carries only a 76% accuracy rate in predicting significant injury on biopsy (288).

Table 39.7. Predictors of More Severe Histology in Nonalcoholic Steatohepatitis

Age >40–50 y or female gender
Degree of obesity or steatosis
Hypertension
Overt diabetes or increased insulin resistance
Hypertriglyceridemia
Elevated alanine transaminase level
Elevated aspartate transaminase level
Elevated γ-glutamyl transpeptidase level
Aspartate transaminase:alanine transaminase ratio >1
Elevated immunoglobulin A level
See text.

Limitations of the Biopsy

The major limitations of biopsy include patient inconvenience, the potential for complications, performance in obese patients, and sampling error. In general, complication rates with liver biopsy are infrequent, and the variety of available techniques offer improved safety and applicability (289,290,291,292). Sampling error on percutaneous biopsy is well recognized but can be minimized by obtaining an adequate specimen more than 2 to 3 cm in length by 1.5 mm wide (15 to 16 gauge) (293,294,295). In the most convincing study, Ratziu et al. demonstrated a 10% to 15% risk of a two-stage error and roughly 40% risk of one-stage error in the fibrosis stage when specimens are less than about 2 to 3 cm in length (Fig. 39.11) (269).

Scoring of the Biopsy (Nonalcoholic Steatohepatitis Activity Index, Nonalcoholic Steatohepatitis Activity Score)

Although the Brunt score remains the most commonly used method of assessing the biopsy, recent introduction of composite scores have provided a useful means of assessing response to treatment particularly in clinical investigations (Table 39.8). Key parameters have been combined into the NAI and NAS (297,298). The NAI ranges from 0 to 12 and accounts for steatosis, necroinflammatory activity, and hepatocyte injury (ballooned cells), each of which is scored from 0 to 4. The more refined NAS uses a scale of 0 to 8

to score steatosis, lobular inflammation, and cellular ballooning. Fibrosis stage is scored separately in both composites.

Table 39.8. Histology Scoring Systems in Nonalcoholic Fatty Liver Disease

	Steatosis	Inflammation (lobular)	Hepatocyte injury (ballooning)	Maximum score	Fibrosis
NAS-I (NAFLD activity score) (Brunt et al., 1999) (23)	0–3	0–3	0–2	8	0–4
	0—none	0—no foci	0—absent		0—none
	1—<33%	1—1–2 foci/mpf	1—present-z3		1—sinusoidal
	2—33%–66%	2—3–4 foci/mpf	2—marked-z3		2—sinusoidal and periportal
	3—>66%	3—>4 foci/mpf			3—bridging fibrosis
					4—cirrhosis
NAI (NASH activity index) (Promrat et al. 2004) (296)	0–4	0–4	0–4	12	0–4
	0—<5%	0—no foci	0—absent		0—none
	1—5%–25%	1—<1 foci/mpf	1—only z3, <50% of CVs		1—perisinusoidal
	2—>25%–50%	2—1 foci/2 mpf	2—only z3, >50% of CVs		2—perisinusoidal and periportal
	3—>50%–75%	3—1–2 foci/mpf	3—both z2 and z3, (1/3–2/3)		3—bridging fibrosis
	4—>75%	4—>2 foci/mpf	4—all zones (>2/3)		4—cirrhosis/regeneration
NAS-II (NASH clinical research network revision) (Kleiner et al., 2005) (297)	0–3	0–3	0–2	8	0–4
	0—<5%	0—no foci	0—absent		0—none
	1—5%–33%	1—<2 foci/mpf	1—few ballooned cells		1—perisinusoidal or periportal 1A—mild, z3, perisinusoidal 1B—moderate, z3, perisinusoidal 1C—portal/periportal
	2—>33%–66%	2—2–4 foci/mpf	2—many/prominent ballooning		2—perisinusoidal and portal/periportal
	3—>66%	3—>4 foci/mpf			3—bridging fibrosis 4—cirrhosis

NAS, NASH activity score, nonalcoholic stestohepatitis; NAI, NASH activity index, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; z2, zone 2; z3, zone 3; mpf, medium power field—200× magnification; CVs, central veins.

Natural History and Prognosis

Mortality Overview

Although patients with NAFLD frequently have substantial comorbid conditions that could influence survival, progressive liver disease often becomes the dominant problem. Consistent with this, obesity was recognized as a major risk factor for cirrhosis-related deaths in people who consume little or no alcohol (299). Among patients with type 2 diabetes in Japan, the cause of death was cirrhosis in 6.4% (compared to 19.5% from heart disease) (300). However, the observed versus expected deaths ratio (O/E ratio) was actually higher for cirrhosis than for heart disease (2.67 vs. 1.81). In another report, the five- and ten-year survival in NASH was estimated at 67% and 59%, respectively (301). These figures were lower than those for a matched population, but the difference did not reach statistical significance. In a 12-year follow-up, Cortez-Pinto et al. showed that patients with NASH had a liver-related death rate similar to that for ambulatory patients with ASH—both rates were significantly better than those for hospitalized patients with ASH (302). In one of the largest natural history studies published to date, Adams et al. showed that liver disease is the third leading cause of death in a group of 420 patients with NAFLD followed up for a mean of 8 years compared to liver disease as the thirteenth most common cause of death in the general population (303,304).

Variation Based on Initial Histology

In a retrospective study of adult patients in the United States, the overall mortality among those with fatty liver accompanied by inflammation, fibrosis, balloon cells, or Mallory hyaline was increased compared to crude death rates, and cirrhosis-related deaths were also increased when fatty infiltration was accompanied by the presence of these more severe histologic markers (7). In contrast, several prior studies have shown that simple steatosis or steatosis with minimal inflammation (Matteoni type 1 and 2) is a relatively stable condition (15,305). The relative stability of these milder forms of fatty infiltration indicates that NASH (fatty infiltration plus fibrosis and/or balloon cells) probably begins at the higher stage rather than progressing through stages from simple steatosis to more severe forms. However, this has not been established and such a transition can be seen with rapid weight loss (and perhaps with other forms of metabolic stress) (306,307).

Serial Biopsy Studies

A number of studies reporting serial biopsy in patients with NASH have now been published (5,25,26,89,308,309,310,311). Although the studies have a number of shortcomings, including variable biopsy techniques, different entry criteria, and incomplete data on confounding variables such as voluntary lifestyle changes and antidiabetic or antihyperlipidemic medications (312), there is sufficient data to draw some reliable observations. Compiling the studies, 177 patients have undergone a second biopsy after a mean of 4.5 years (Table 39.9). Cirrhosis developed in 10% of patients while fibrosis progressed in 33%, remained stable in 41%, and improved in 22%. In some of the patients, the progression to cirrhosis was surprisingly rapid over 1 to 2 years. Most papers show that as fibrosis progresses, aminotransferases, steatosis scores, and inflammation improve paradoxically. This is of some concern in the clinical setting in which normalization of the aminotransferases should be regarded with cautious optimism, especially in older patients. Normalization of these parameters is consistent with the progression of NASH to a "burned out" state, which is often recognized as "cryptogenic" cirrhosis (see preceding text).

Table 39.9. Serial Biopsies in Patients with Cryptogenic Cirrhosis

Author	Number	Years of follow-up	Progressed to stage 4	Progressed to stage 2–3	No change	Improved
Lee (1989) (308)	12	3.5	2	3	7	0
Powell (1990) (24)	13	4.5	3	3	6	1
Bacon (1994) (5)	2	5	1	0	1	0
Ratziu (2000) (89)	4	5	1	1	2	0
Harrison (2003) (308)	19	5.7	2	4	9	4
Fassio (2004) (309)	22	4.3	0	7	11	4
Adams (2005) (310)	98	3.2	9	24	35	30
Totals/Average	177	4.5	18 (10%)	59 (33%)	73 (41%)	39 (22%)

Nonalcoholic Steatohepatitis–Related Cirrhosis and Cryptogenic Cirrhosis

NASH-related cirrhosis appears, like other forms of cirrhosis, to progress through several stages. A small number of studies have addressed the natural history of this form of cirrhosis. Hui et al. compared the course of 23 patients with well-defined NASH cirrhosis to 46 matched patients with cirrhosis from hepatitis C (313). Nine of 23 patients with NASH–cirrhosis developed major complications of portal hypertension (e.g., ascites, encephalopathy, and variceal bleeding) during a mean follow-up of 7 years, with complication-free survival of 83%, 77% and 48% at 1, 3 and 10 years, respectively. Ratziu et al. compared the course of 27 overweight patients with cryptogenic cirrhosis to 10 lean patients with cryptogenic cirrhosis and 391 patients with hepatitis C–related cirrhosis in a retrospective follow-up cohort study (314). With a mean follow-up of 22 months, 2 of the 15 patients presenting only with abnormal liver test results developed major complications of portal hypertension and 5 developed HCC. The overall severity and risk for either a complication of portal hypertension or HCC were greater in obese patients with cryptogenic cirrhosis compared to the lean cryptogenic cirrhosis group but not different from patients with hepatitis C. The authors concluded that obesity-related cirrhosis behaves as aggressively as hepatitis C–related cirrhosis.

Hepatocellular Cancer

A number of studies have now shown an increased risk of HCC in obese patients and in those with diabetes (315,316,317,318,319). In addition to the natural history studies noted in the preceding text, there are also a number of well-documented NASH case reports and series indicating this progression (320,321,322). Although animal models of fatty liver exist in which HCC develops without cirrhosis, most human studies suggest that silent progression of NASH to cirrhosis is the more predominant pathway (323,324). The association of NASH with cryptogenic cirrhosis (see preceding text) and the risk of HCC in cryptogenic cirrhosis further strengthens the idea that for many patients with progressive NASH, HCC is an increasingly common late complication (325,326,327). Although the molecular events leading to HCC are yet to be fully defined, the proliferation of oval cells (hepatocyte progenitor cells) observed in human and experimental NAFLD may be a contributing factor (328) in addition to mutations in regulator genes such as *PTEN* that regulate certain aspects of both fat metabolism and cell proliferation (329).

Experimental and Animal Models of Nonalcoholic Fatty Liver Disease

Small Animal Models

Many advances in the understanding of NAFLD have resulted from the development of small animal models—usually mice or rats. This field has been reviewed extensively by Koteish and Diehl (330), Farrell (331), and Nanji (332). Several of the best known models include

the hyperphagic ob/ob mouse, which has a congenital deficiency of leptin; the FA/FA rat, which has an impaired leptin receptor; and the methionine–choline–deficient (MCD) rodent. Studies in these animals, as well as the numerous transgenic models, have led to many seminal observations in NASH about hepatic fat metabolism and its regulation. Although providing insight into specific pathways, these models share the common problem of inadequately imitating the common form of human NAFLD. For example, the ob/ob mouse requires other provocative measures to produce significant injury in addition to simple steatosis, and the MCD model, although producing hepatic injury without further provocation, lacks insulin resistance (333).

Large Animal Steatosis and the Liver as a Normal Fat-Storing Organ

Fatty liver disease is a well-known problem in veterinary medicine. Variants of the disorder are seen in cows (334,335,336), hens (337), and cats (338,339) and can occur spontaneously or with phosphorous supplements in pigs (340,341,342). Hepatic "lipidosis" is reported to be one of the most common liver disorders in domestic cats and, similar to human steatohepatitis, has been associated with mitochondrial morphologic abnormalities (343). Seasonal variation in hepatic fat has been observed in deer (344). These observations suggest a close integration of the liver into the adipose system, which is evident experimentally in studies demonstrating the upregulation of genes governing adipocyte differentiation during liver regeneration (345,346).

Palmipedes (migratory geese) develop fatty liver before migration and utilize fat as a preferred source of energy for muscles through the expression of fatty acid–binding protein (347). This has been exploited in the production of foie gras in which geese are fed a corn-based diet, resulting in a 8- to 10-fold increase in liver size in as little as 2 weeks (from 100 to 800 g). The goose hepatocyte enlarges to three to four times the original diameter, with fat droplets observable as mixed micro- and macrosteatosis. Although early harvest limits the natural course, degenerative changes

is seen and cirrhosis is anecdotally noted (348). In addition, accidental exposure to moldy corn (mycotoxins) is associated with liver failure with ascites (349). Interestingly, significant subspecies variation exists in the lipogenic capacity (350). Indirect evidence suggests that the main mechanism involves increased lipid synthesis and altered very low density lipoprotein (VLDL) synthesis and secretion (351,352,353). These observations further illustrate the role of the liver within a broadened concept of the adipose system, with its inherent plasticity (transdifferentiation of fat stores) and its role in energy metabolism and thermoregulation (354,355,356,357). It has been hypothesized that skeletal muscle fat metabolism, which interacts with hepatic fat stores and strongly influences insulin sensitivity, confers a survival advantage under harsh conditions (358).

Pathogenesis of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

Although many of the metabolic abnormalities noted in NAFLD have yet to be assimilated into a cohesive "story," the importance of lipotoxicity as underlying insulin resistance, the metabolic syndrome and cellular injury in steatohepatitis, is emerging as the most likely primary pathway in typical cases of human NAFLD (359). In this light, NAFLD can be viewed as a potential adverse occurrence within the realm of the metabolic syndrome and more specifically as a possible adverse outcome of systemic lipotoxicity (360). Excessive fatty acids have a broad effect on many tissues and even influence gene expression and, hence, adipocytokine production in adipose tissue (361). However, it is very likely that there is substantial variation between groups or individuals in the relative importance of different mechanisms, as suggested in the wide ethnic variation in the development of steatosis—the first "hit" in the path to hepatic fibrosis. A plausible pathway for some of the most common abnormalities is shown in Figure 39.12.

Steatosis

The normal, healthy liver contains no more than 5% lipid by weight (362). The levels of both triglycerides (mostly unsaturated fatty acids) and free fatty acids (mostly saturated) are increased in the liver of obese patients (363). The development of cellular injury involves a cascade of events beginning with the development of steatosis (NAFLD) and the subsequent development of oxidative stress, lipid peroxidation, and cell injury, and activation of profibrotic cytokines, resulting in NASH (364). Increased hepatic fat stored in the form of triglyceride can be derived from the plasma fatty acid pool mostly released from peripheral adipose tissue by lipolysis, from dietary fat through chylomicron remnants, or from de novo synthesis within the liver. De novo lipid synthesis, largely from glucose, is governed by two main transcription factors that signal transcription of the enzyme systems responsible for fatty acid synthesis and subsequently for their esterification into triglyceride: Sterol regulatory element–binding protein (SREBP), which is governed by insulin, and carbohydrate response element–binding protein (CREBP), which is governed by glucose levels (365,366,367,368). SREBP-1 level appears to be increased in animal models of NAFLD, and it is suspected that a similar process is going on human NAFLD.

The disposition of fatty acid in the liver proceeds by one of several routes: Storage as triglyceride, export as VLDL, or oxidation. Regulation of the predominant form of disposition depends on a number of interacting factors based on energy homeostasis and influenced by peroxisome proliferators activated receptor (PPAR) activity and probably by the activity of the adrenergic nervous system (369,370,371). Although focal fat necrosis may occur from direct release of fat from swollen hepatocytes in NAFLD (372), toxicity, as noted by Bass and Merriman, results primarily from the indirect effects of lipid peroxidation and to a lesser extent from direct toxicity of fatty acids (369).

Insulin Resistance

Although epidemiologic work indicates that peripheral insulin resistance is neither sufficient nor essential for NAFLD, it is present in most patients with NAFLD and, therefore, is so closely intertwined with the disease as to be virtually inseparable in most cases. Insulin resistance is characterized by a reduced sensitivity to insulin in target tissues (e.g., muscle, adipose tissue, and liver), where it normally favors glucose transport into the cell, storage of glycogen, inhibition of lipolysis in adipose tissue, and inhibition of gluconeogenesis from the liver. The expected manifestations of insulin resistance include decreased peripheral (muscle) glucose utilization, enhanced lipolysis and mobilization of fatty acids from peripheral fat stores, and increased hepatic glucose output (normally suppressed by insulin). Insulin resistance, primarily mediated by excessive fatty acids (373,374,375,376,377,378,379), is observed in NAFLD using a variety of techniques (112,380,381,382). The relationship between fatty liver and insulin resistance represents a (sometimes precarious) balance between the three main target organs—skeletal muscle, adipose tissue, and the liver (383). Using modifications of the insulin "clamp" test, Sanyal et al. convincingly demonstrated that the predominant site of resistance in NAFLD is in the peripheral fat and skeletal muscle as opposed to the liver (94,384,385,386,387). Recent work using MRS of skeletal

muscle indicates that insulin resistance in metabolic syndrome results from skeletal muscle lipotoxicity and secondary changes in mitochondrial metabolism (388). Excessive skeletal muscle fatty acid also leads to inhibition of insulin-stimulated glucose transport through the effects on insulin receptor substrate-1 (IRS-1) (389). Teleologically, insulin resistance in this setting can be seen as a normal response to excessive energy substrate availability (diet and obesity) and underutilization (activity).

Lipid Peroxidation and Hepatic Lipotoxicity

Lipid peroxidation reflects an imbalance between pro- and antioxidant substances (oxidative stress) (390). It is a branching, chain reaction stimulated by a free radical attack on unsaturated fatty acids (Fig. 39.12) (391). Free radicals, which initiate the process, may be derived from mitochondrial, peroxisomal, or cytochrome P-450 fat metabolism, with the formation of superoxide, hydrogen peroxide, and hydroxyl radicals. The products of the reaction are another free radical and a lipid hydroperoxide, which, in a reaction catalyzed by iron, forms a second (lipid) free radical and, therefore, amplifies the process. Damage involves chemical bonding with other cellular constituents including membrane lipids, proteins, and DNA (392). Although difficult to measure directly, lipid peroxidation is the main process leading to inflammation, activation of cytokines, stimulation of stellate cells, and fibrosis (393,394). Levels of the markers of

lipid peroxidation (e.g., nitrotyrosine, 4-hydroxynonenal [4-HNE], and malonic dialdehyde [MDA]) are increased in human NASH and are associated with mitochondrial abnormalities (94,395,396). The latter may be an injury response or due to increased activity of uncoupling protein (UCP), which decreases oxidative stress and is associated with abnormal oxidative phosphorylation and an adenosine triphosphate deficit (94,270,397,398,399,400). Cytokine level elevation activates stellate cells with secondary fibrosis. Interestingly, a major site of oxidative injury appears to be the border area of small fat droplets that consist of a unique phospholipid monolayer (401,402).

Cytokine Activation and Fibrosis

Cytokine level elevation, especially tumor necrosis factor-α (TNF-α), has been well described in NAFLD (403). In animal models, obesity itself appears to sensitize the liver to cytokine-mediated injury (404,405), TNF-α has been shown to induce mitochondrial UCP in regenerating liver in animal models of nonalcoholic fatty liver, and transforming growth factor-β (TGF-β) and interleukin-6 (IL-6) have also been implicated as mediators of fibrosis in NASH (403,406,407). Cell culture experiments indicate that stimulation of TNF-α results from fatty acid–mediated destabilization of lysosomes (408). Indirect evidence suggests that lipoperoxide-induced expression of inflammatory cytokines is mediated by the transcription factor nuclear factor-κB (409). A number of additional transcription factors (e.g., PTEN) and cytokines (e.g., osteopontin) are being recognized that, at least in experimental conditions, appear to interact with each other and influence insulin signaling and fibrosis pathways (410,411,412).

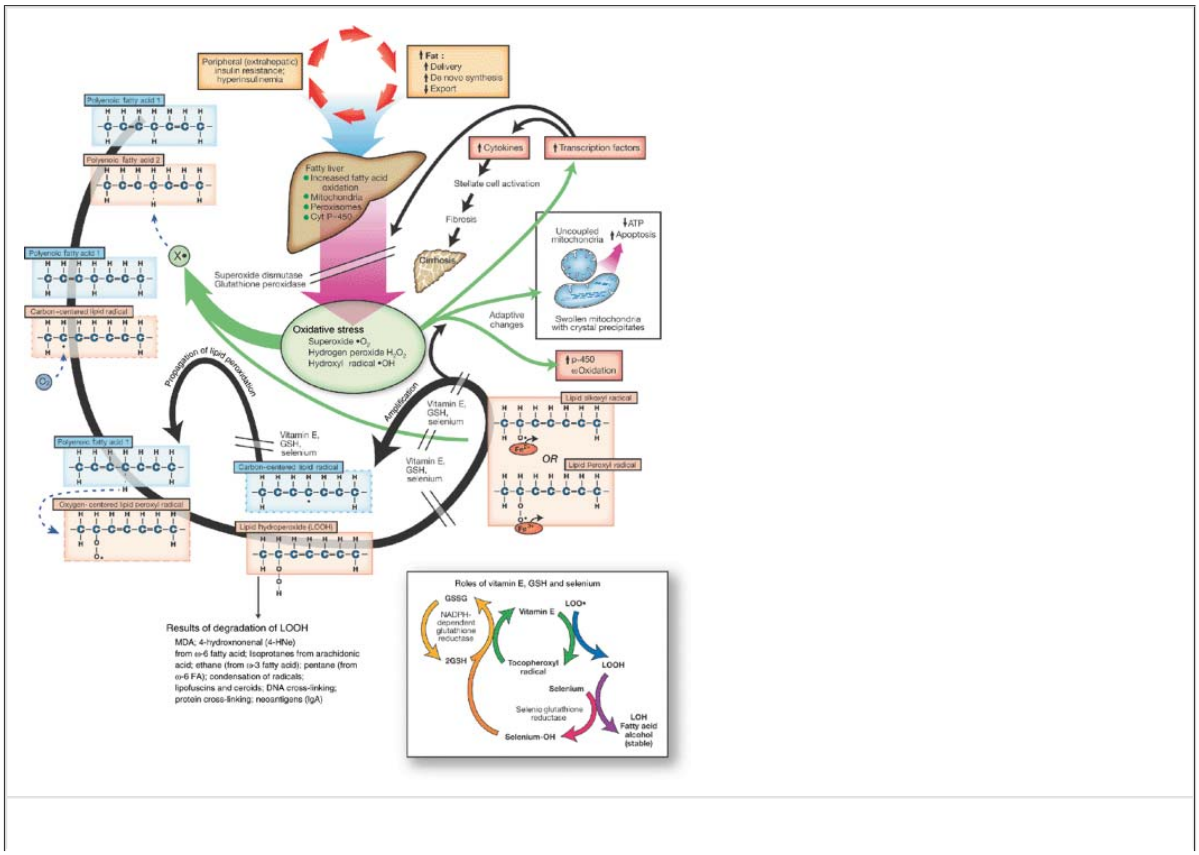
Adiponectin and Leptin (Adipocytokines)

Adiponectin is the most abundant protein in the adipocyte and participates in glucose homeostasis and insulin signaling through receptors in the muscle (adipoR1) and liver (adipoR2) (413,414,415,416,417). Its level is decreased in both obese/diabetic mice and humans and in patients with NASH compared to body fat–matched controls (416,418,419). Unlike leptin (see subsequent text) or TNF-α, adiponectin levels appear to be significantly different in patients with simple steatosis versus those with NASH, suggesting a more significant role for it in disease pathogenesis including a possible anti-inflammatory effect (420,421,422,423). Leptin is a circulating protein coded for by the obesity gene (chromosome 7q31 in humans) and produced primarily in white adipose tissue and its level is increased in cirrhosis (424,425,426,427). Its primary role is to govern satiety through action at the hypothalamus; however, human obesity is usually associated with elevated leptin levels (428). It has been variably implicated in the development of histologic injury in human and experimental NAFLD (429,430,431,432,433). In a recent study from Angulo et al. elevated leptin levels in progressive NASH were attributed to factors involved in production; no difference in leptin was seen between patients with worsening injury or those without on serial biopsy (434).

Other Cellular Injuries (Ballooning and Apoptosis)

Although the ballooned hepatocyte constitutes a marker for more progressive injury, a consensus definition remains elusive. The normal diameter of the hepatocyte varies from 13 to 30 μm (435). On light microscopy, ballooned cells are described as 1.5 to 2 times the normal size, located predominantly in zone 3 compared to other zones, and have rarefied cytoplasm. Ballooning in viral hepatitis involves hydropic changes and irregular dilatation of the smooth endoplasmic reticulum (436,437). However, electron microscopy using osmium fixation, which highlights fat droplets, suggests that most such cells in NASH

are microsteatotic (438). Coupled with observations of localization of lipid peroxidation (see preceding text) and localization of Mallory bodies (439), these observations suggest that handling of small-droplet storage fat is a significant source of injury in NASH. Apoptotic bodies are only occasionally seen in NASH specimens, although signal pathways for apoptosis are significantly activated in human NASH (440). This paradoxical situation is the result of rapid "cleanup" of apoptotic bodies such that they are infrequently seen or it may represent the result of a (sometime precarious) balance of pro- and antiapoptotic factors involving adaptive changes in the mitochondrion that play a central role in the regulation of apoptosis (441,442,443). Such adaptive (or maladaptive) changes may make the fatty liver more resilient in terms of localized cell death but more prone to necrosis because these processes are closely interrelated and coregulated (444).



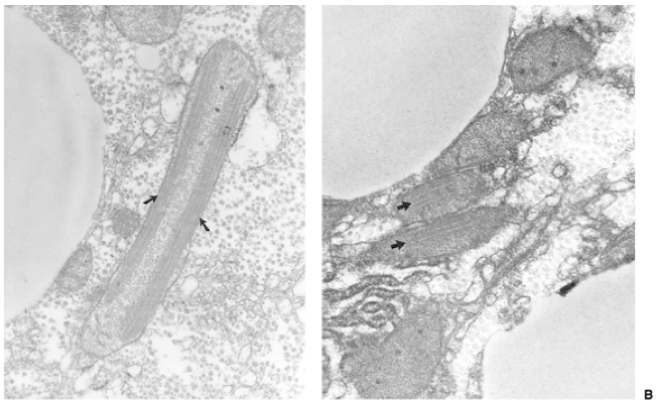
• **Figure 39.12** Mechanism of steatohepatitis—increased hepatic fat stores result from increased delivery of fatty acids, increased de novo synthesis, and decreased export of fat. The excessive free fatty acids both result from and promote extrahepatic insulin resistance. Fatty acid oxidation leads to the formation of free radicals, which derive from mitochondrial, peroxisomal, and cytochrome P-450 oxidation. Free radicals (e.g., super oxide, hydrogen peroxide, and hydroxyl radical) can directly activate transcription factors, resulting in overexpression of cytokines and, if not neutralized by the antioxidant system (superoxide dismutase and glutathione peroxidase), the free radicals ($X^{\bullet}$) can trigger the chain reaction of lipid peroxidation (rancidification). Unsaturated, polyenoic fatty acids are especially susceptible. This produces a *carbon-centered lipid radical*, which reacts with oxygen to form an *oxygen-centered lipid peroxyl radical*. This substance subsequently reacts with a second fatty acid to form another lipid free radical (propagation) and a *lipid hydroperoxide*. The latter is unstable and, in the presence of iron and another fatty acid, reacts to form yet another lipid radical (amplification). Alternatively, the lipid hydroperoxide may degrade to malonic dialdehyde (MDA) (detected by the thiobarbituric acid reactant [TBAR] reaction) or to ethane or pentane (detected in breath tests), react with other radicals to form a stable pigment (e.g., ceroids and lipofuscins), or may cross-link with deoxyribonucleic acid (DNA) or other cellular proteins (neoantigen formation). This oxidative stress induces cytochrome P-450 fatty acid oxidation and causes mitochondria changes with enlargement and formation of crystalline bodies possibly a result of abnormal expression of uncoupling protein. This leads to an adenosine triphosphate (ATP) deficit that increases the risk of necrosis and probably stimulates apoptosis, resulting in sporadic cell death and increased cell turnover. Lipid peroxidation also causes transcription of profibrotic cytokines that activate stellate cells, producing fibrosis. Countering the process of lipid peroxidation is the antioxidant system, which neutralizes lipid radicals by combination with vitamin E. The latter is then restored by shuffling the radical groups to glutathione through selenium. GSH, glutathione; NADPH, nicotinamide adenosine dinucleotide phosphate, reduced form; GSSG, oxidized glutathione; IgA, immunoglobulin A.

Mitochondrial Changes and Adenosine Triphosphate Homeostasis

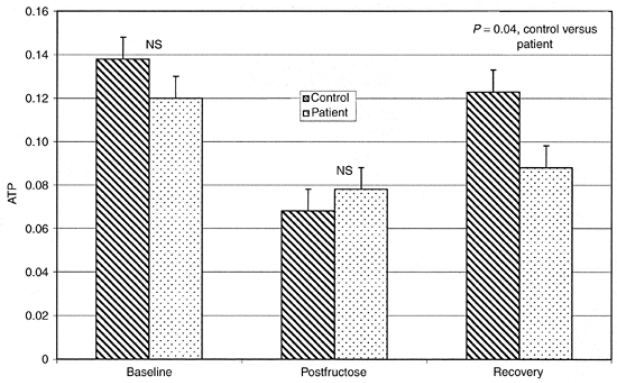
The mitochondrion may be both an especially important source of reactive oxygen species and a target for injury resulting from lipid peroxidation (196,445,446). Its unique evolutionary history places it in a central position of several major metabolic pathways, including fatty acid synthesis and β-oxidation, oxidative phosphorylation (ATP generation), and signaling pathways for the process of apoptosis (447). Mitochondrial morphologic abnormalities (Fig. 39.13) have been observed in both ASH and NASH (94,229,448,449,450). Commonly noted intramitochondrial inclusions are thought to be either a protein or phospholipid precipitate (451). It is thought that these morphologic abnormalities correlate with functional abnormalities, including respiratory chain dysfunction, and by their distribution may be part of the adaptive process to oxidative stress that make the liver more tolerant to reactive oxygen species but more susceptible to ischemic injury (398,452,453). For example, the poor function of steatotic livers in transplantation has been attributed in part to abnormal ATP homeostasis with depletion of electron transport components and increased susceptibility to ischemic injury (454,455,456,457).

Impaired function of the mitochondrial electron transport chain (ETC) in NAFLD has been described in several studies (458,459), attributable in part the to overexpression of UCP induced by increased fatty acids (460). Perez-Carreras et al. noted reduction in the ETC activity to 40% to 70% of normal in all the major complexes (I to V) in human NASH. In vivo impairment of ATP synthesis was observed by Cortez-Pinto et al. using ^{31}P MRS of the liver in controls compared to patients

with NAFLD (Fig. 39.14) (270). In experimental conditions, complex I and V (ATP synthase) dysfunction have been implicated in reperfusion injury, and these results open inquiry as to what the optimal protective mechanism is for the ETC during liver transplantation. (461,462). In a recent exhaustive review of the subject, Pessayre and Fromenty proposed that NASH is primarily a "mitochondrial disease." Although this may initially seem to be an overstatement, a cogent argument is presented on the fundamental role of the mitochondrion in hepatic fat metabolism, skeletal muscle physiology, insulin resistance, and pancreatic islet cell vitality, placing the mitochondrion at a central point in the overall pathophysiology of NAFLD (463).



• **Figure 39.13** Deformed mitochondria with crystalline inclusions in nonalcoholic steatohepatitis: **A:** An elongated mitochondrion with two bundles of parallel, crystalline structures (*arrows*) near a large fat droplet. **B:** Two mitochondria cut tangentially showing crystalline inclusions in an area of cytoplasm between two large fat droplets (*arrows*). Note the paucity of normal cristae.



• **Figure 39.14** Abnormal adenosine triphosphate (ATP) homeostasis in nonalcoholic fatty liver disease. Initial fructose metabolism requires the expenditure of energy in the form of ATP. Intravenous injection of fructose normally produces a significant drop in hepatic ATP stores, as measured by magnetic resonance spectroscopy, followed by return to baseline. The baseline in patients with fatty liver is similar to that in healthy controls, but there is a blunted recovery indicating the presence of a relative energy deficit in the fatty liver. NS, not significant. (Adapted from Cortez-Pinto, Chatham J, Chacko VP, et al. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* 1999;282:1659–1664.)

Cytochrome P-450

Similar to alcohol-related liver disease, induction of cytochrome CYP 2E1 in NAFLD has been described as a possible source of oxidative stress and activation of cytokines (464,465,466) through enhanced microsomal ω-oxidation of fatty acids (normally a minor pathway of fatty acid metabolism). Expression of CYP 2E1 is influenced by a high-fat, low-carbohydrate diet and colocalizes in immunohistochemical stains to fatty cells and markers of lipid peroxidation (467,468). It is associated with increased activity of mitogen-activated protein kinases (MAPKs) through the activity of extracellular signal–regulated kinases 1 and 2 (ERK1/2), which participate in the regulation of cell death (apoptosis) pathways (469). On the basis of animal studies, it is unlikely that P-450 2E1 plays a singular role in NASH because CYP 2E1 nullizygous, transgenic mice also develop a steatohepatitis-like picture (470). Indeed, Schattenberg et al. (469) showed experimentally that overexpression of CYP 2E1 is protective against oxidative injury and decreases apoptosis but increases the risk of necrosis induced by fatty acid exposure. Consistent with this adaptive process, inhibition of CYP 2E1 in experimental conditions increases formation of Mallory bodies (471).

Abnormal Lipoprotein Metabolism

Consistent with its close association with hyperlipidemia and metabolic syndrome, NASH has been associated with abnormal apolipoprotein (apo) metabolism (472). Two groups have reported decreased apoB-100 secretion in NASH, indicating possible impairment of VLDL synthesis (a major component of which is apoB-100) (473,474), and another group has described differences in apoA-I (a component of HDL) in patients with NAFLD compared to controls (475). Isolation of small lipid droplets from hepatocytes of rats in experimental studies has demonstrated that the lipid droplets

are composed of neutral fats consisting mainly of esters of linoleic, oleic, and palmitic acid (24). Similar studies in human liver biopsy samples from patients with ASH have shown that the lipid composition of accumulating lipid droplets is similar regardless of size but that the smallest droplets, based on size, density, and lipid composition, resemble a precursor of plasma VLDL (476,477). Taken together with observations of cellular ballooning (see preceding text), these observations again point to the abnormal handling of the small storage lipid droplets as being significant in NASH progression.

Peroxisomal Metabolism

The peroxisome is involved in numerous metabolic pathways including synthesis of plasmalogens, bile acids, and cholesterol, and oxidation of very long chain fatty acids, branched-chain fatty acids, dicarboxylic acids, polyunsaturated fatty acids (PUFA), L-pipecolic acid, and phytanic acid (478,479). Steatohepatitis develops in mice lacking peroxisomal fatty acyl-CoA oxidase (480), and morphologic abnormalities with diminished size but increased number of microsomes have been described in human fatty liver (481). Peroxisomal fatty acid oxidation represents another potential source of reactive oxygen species including superoxide and hydrogen peroxide that form during peroxisomal oxidation of very long chain fatty acids and metabolism of dicarboxylic acids (derived from cytochrome P-450 ω-oxidation of very long chain fatty acids) (482). Multiple inherited disorders of peroxisomal metabolism have been described including Zellweger's syndrome, adrenoleukodystrophy, and Refsum's disease. Although disturbed peroxisomal metabolism does not appear to be a primary factor in most patients with NASH (based on normal levels of dicarboxylic acid (94) and normal very long chain fatty acid profiles in patients with NASH—Caldwell et al.), it is possible that genetic abnormalities, nutritional abnormalities, or adaptive changes in the peroxisome may contribute to the condition in some patients.

Other Conditions Associated with Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis—"Secondary" Nonalcoholic Steatohepatitis

The foregoing discussion has largely centered on what some refer to as "primary" NASH or NAFLD typically associated with insulin resistance and the metabolic syndrome. The presence of other factors may suggest a separate disease with overlapping features of NASH (Tables 39.1 and 39.4) distinguished primarily by the lack of insulin resistance. However, this does not exclude the likelihood that many such patients have underlying "primary" NASH exacerbated by some other insult.

Bariatric Surgery

Historically, weight-reduction therapy played an important role in the recognition of NAFLD/NASH because of the unexpected exacerbation that was noted in some patients after early jejunoileal bypass (483). Stimulation of TNF by bacterial endotoxin has been postulated as an etiologic factor, but this was not supported by efforts to prevent injury using oral antibiotics (484) or by reports of a similar process after gastroplasty, in which bacterial overgrowth is less of a problem (485). Micronutrient deficiency has also been proposed. The rate of weight loss may be a key factor by increasing the rate at which intra-abdominal fat is mobilized. In spite of its historical association with exacerbation of NASH, weight loss surgery (particularly gastric bypass) remains a viable option in some patients (see subsequent text).

Medication Induced

A number of medications have been implicated as causes of steatohepatitis. In several cases, particularly with nifedipine and diltiazem (486,487), the association may be one of common drug use in patients at high risk for NASH. Similarly, Chitturi and Farrell pointed out in a recent paper that the risks for methotrexate-induced steatohepatitis are almost identical to those of NASH, suggesting a high degree of overlap between these entities (466,488). Tamoxifen-induced steatohepatitis presents a particularly difficult balance of risk–benefit in patients on therapy for prior breast cancer (489,490). Although liver injury was not considered a side effect in a recent review of adjuvant breast cancer therapy (491), the authors and others have observed severe steatohepatitis with cirrhosis in this setting (492,493,494). The risk factors for tamoxifen-induced steatohepatitis are, as with methotrexate, similar to those of NASH, suggesting a possible synergistic effect of the drug in a patient prone to NASH. Amiodarone, which has long been associated with phospholipidosis and steatohepatitis (495,496,497), has become an extremely common medication in cardiology. As with tamoxifen, its use should not be undertaken without some consideration of the high likelihood that many recipients will have preexisting fatty liver because of shared risks between NASH and heart disease. The issue again becomes one of risk versus benefit. We have observed advanced cirrhosis with death due to

liver failure in this setting and associated litigation. To our knowledge, an adequate risk–benefit analysis has not been done.

Human Immunodeficiency Virus Therapy

An acquired lipodystrophy, associated with insulin resistance and steatosis and sharing features with multiple symmetrical lipomatosis, has been described with nucleoside analog therapy for human immunodeficiency virus (HIV) infection (on highly-active antiretroviral therapy [HAART]) (498,499,500,501). The syndrome may be increased in women, may present acutely, and can be associated with a Reye's syndrome–like picture, neuropathy, myopathy, and pancreatitis (502). There are increased concerns that patients with this syndrome are at increased risk of developing NAFLD and the possibility of progression to NASH and cirrhosis. The pathogenesis of NAFLD from HAART therapy is related to insulin resistance (383), mitochondrial DNA damage (503), and the development of lactic acidosis (504).

Parenteral Nutrition, Malnutrition, and Celiac Disease

Liver disease, often with macro- and microvesicular steatosis, is one of the most common and potentially severe side effects of total parenteral nutrition (TPN) (505). In one series of patients on extended TPN, macrovesicular steatosis was seen in 63% of patients with cholestasis and 100% of those without cholestasis (506). Microvesicular steatosis and phospholipidosis (fat-laden cells in the sinusoidal space or portal tract) are also common features. Both the amount of lipid infusion and its composition appear to affect the expression of liver disease in this setting (507). Choline deficiency may play a role in some patients. At the opposite end of the nutrition spectrum, fatty liver is a common finding in kwashiorkor, in which export of lipid from the liver because of protein deficiency (diminished apoprotein B) is thought be the primary mechanism (508). In both types of nutritional fatty liver, zone 1 (periportal) involvement may predominate. There have been reports suggesting an association between NASH/NAFLD and celiac disease (509,510,511). Bardella et al. examined 59 consecutive patients with elevated transaminase levels and NAFLD and found that 6 patients had positive tissue transglutaminase antibodies and 2 (3.4%) were positive for antiendomysial antibodies. Overall, two patients (3.4%) were positive for both antibodies and had positive histology (512). In another report, Nehra et al. observed that 1 out of 47 patients with NASH was positive for antiendomysial antibodies (514).

Solvents and Industrial Agents

A variety of toxins have been implicated in the development of fatty liver diseases (514). Better described agents include carbon tetrachloride (now rarely used), dimethylformamide, perchloroethylene, and petrochemical derivatives (515,516,517,518). Other compounds and elements that have been implicated include phosphorous (See "Experimental and Animal Models of Nonalcoholic Fatty Liver Disease"), ethyl bromide, ethyl chloride, and rare earths. Synergy between exposure to these agents and disease progression in an obese patient and/or a patient with diabetes is suspected but not established. Cotrim et al. have demonstrated a potentially progressive form of NAFLD that results from petrochemical exposure and occurs in the absence of insulin resistance (519).

Wilson Disease

Macro- and microvesicular steatosis are well-known features of Wilson disease (520,521). Consideration of Wilson disease should be made especially with steatohepatitis in a younger individual. It is not known how often the carrier state for mutations in the nuclear-encoded gene for copper-transporting ATPase (522) could play a role in more typical cases of NASH. We have noted borderline values of ceruloplasmin occasionally in patients with some features of obesity-related steatohepatitis (S. Caldwell, unreported clinical observation, 2004). Mitochondrial injury, mutations, and premature oxidative aging were recently described in patients with Wilson disease, suggesting a possible overlap (through mitochondrial dysfunction) with more typical NAFLD and NASH (523).

Inherited Metabolic Diseases

Macrovesicular steatosis can be seen in a variety of inherited metabolic diseases, most, but not all, of which present in childhood. Disorders include glycogen storage diseases (524), galactosemia (525), tyrosinemia (526), heterozygous hypobetalipoproteinemia (527,528), and abetalipoproteinemia. Both of the latter disorders are characterized by impaired formation of VLDL due to decreased synthesis of apolipoprotein B. A number of lipid storage diseases, (e.g., cholesterol ester storage, Niemann-Pick disease, Tay-Sachs disease, and Gaucher's disease) can have excessive fatty infiltration of the liver with cholesterol esters, sphingolipids, phospholipids, sphingomyelin, gangliosides, or glucocerebrosides. Presentation as systemic diseases in infancy (although not exclusively so) and the distribution (predominantly in the reticuloendothelial cells) distinguish the lipid storage disorders from typical NAFLD/NASH (21,529).

Managing the Obese Patient with Diabetes and Cirrhosis

Silent progression of NASH to cirrhosis, often in association with normalization of the liver enzymes, has led to the common situation in which otherwise stable patients with type 2 diabetes are found incidentally to have cirrhosis (299,312). Although early data indicates that therapy aimed at diabetes may ameliorate steatohepatitis (See "Antidiabetic Agents"), there is little knowledge on the effects of other commonly employed medications such as antidepressants, sulfonylureas, HMG-CoA reductase inhibitors, or even insulin itself. Moreover, other adjunct treatments that are commonly used in these patients (e.g., aspirin for coronary disease or angiotensin-converting enzyme inhibitors for prevention of diabetic kidney disease) may have adverse effects if cirrhosis has developed (530). This situation arises because of the insidious development of the hyperdynamic state of cirrhosis as a result of portosystemic shunting. One of the hallmarks of this striking change in physiology is systemic vasodilatation with associated changes in renal hemodynamics. Therefore, in addition to considering disease-specific conditions such as the possibility of varices or HCC, broad treatment considerations need to include a reconsideration of certain tenets of diabetes management. Unfortunately, this aspect of NAFLD has not been adequately investigated.

Treatment of Nonalcoholic Fatty Liver Disease

Who Should be Treated?

Patient selection and the relative risk–benefit of different interventions remain one of the most challenging aspects of treating NAFLD. Although less severe forms of NAFLD, such as simple steatosis or steatosis with only inflammation (types 1 and 2 NAFLD), may progress to cirrhosis, most studies support an increased risk of progression, mainly in the presence of more severe histology at baseline such as ballooned cells and fibrosis (NASH or types 3 and 4 NAFLD) (7,15,305,309,531,532). These data indicate the need for careful patient selection in studying the effects of as yet unapproved pharmacologic interventions (Table 39.10). In general, there is a consensus that dietary changes and increasing activity are cornerstones and that these lifestyle changes are typically part of standard recommendations in spite of limited data and variable acceptance by the patient (see subsequent text). Voluntary adoption of these recommendations complicate the interpretation of pharmacologic therapy—most of the existing publications have lacked controls and, although most have included anthropometric indices such as weight and some have accounted for dietary changes, none has accounted for the degree of conditioning that could influence steatosis and may not be reflected in anthropometric measurements.

Endpoints of Therapy

The primary endpoint of therapy remains changes in the histology (See "Scoring of the Biopsy (Nonalcoholic Steatohepatitis Activity Index, Nonalcoholic Steatohepatitis activity score)"). However, sampling error, especially with cores less than 2 cm, is a potential problem and few of the studies reviewed in the subsequent text have provided sufficient details of the biopsy to account for this confounding variable (269,293,294,295). Novel markers of histologic injury especially applicable to the research setting include stains for lipid peroxide by-products, electron microscopy to assess mitochondrial morphology, and markers of stellate cell activation (557). The major surrogate markers for liver injury include the serum aminotransferase levels (approximation of inflammatory activity), imaging to assess hepatic fat content (e.g., ultrasonography, CT scan, MRI, MRS), and serologic fibrosis panels that are in development. Other important measures include anthropometric indices (e.g., weight, BMI), the degree of physical conditioning (e.g., lactate threshold), measures of insulin signaling (e.g., glucose tolerance testing, HOMA or Quicki, insulin clamp tests), serologic or urinary markers of lipid peroxidation (e.g., malondialdehyde or hydroxynonenal), and cytokine levels such as TNF-α, TGF-β, and adiponectin.

Initial Intervention

Lifestyle changes remain a cornerstone of initial management (558,559,560). Optimistically, diet modification and exercise can be accomplished in obese patients, with as many as 80% of patients achieving dietary goals and 36% achieving exercise goals (561). Pessimistically, even intensive counselling to reduce fat intake (<30% of daily calories) and engage in regular physical activity produces only a modest 5% sustained weight loss (562,563,564). In NAFLD, surprisingly little is known about the effects of specific diet types or how much exercise should be recommended or how best to convey this advice.

Exercise Alone

Visceral adiposity and steatosis have been shown to correlate inversely with the degree of cardiorespiratory fitness (114,565). However, the relative benefit of

exercise *without* weight loss (i.e., the "fit fat" individual) versus aggressive dieting has not been extensively explored in NAFLD (566). Exercise affects insulin signaling primarily through its effect on skeletal muscle substrate utilization and mitochondrial oxidative phosphorylation (567,568). The most effective "conditioning" exercise is the one that just passes beyond the lactate threshold—a level usually associated with some degree of discomfort (569). A number of studies have shown that increasing activity reduces expression of the metabolic syndrome, suggesting that a similar effect might be found with NAFLD (570,571). However, even trained athletes who are obese and with high calorie intake (e.g., Sumo wrestlers) have increased features of metabolic syndrome, indicating a limited benefit of exercise without concomitant weight loss (572).

Table 39.10. Pharmacologic Treatment of Nonalcoholic Fatty Liver Disease/Nonalcoholic Steatohepatitis

Authors	*N*	Design	Agent	Daily dose	Duration	Transaminases	Histology	Hepatic fat by imaging
THIAZOLIDINEDIONES								
Caldwell et al. (2001) (213)	10	Open label	Troglitazone	400 mg	3–6 mo	Improved	Improved mild inflammation	Not evaluated
Acosta et al. (2001) (533)	8	Case series	Pioglitazone	Variable	2–12 mo	Improved	Improved	Not evaluated
Sanyal et al. (2004) (534)	10	RCT	Pioglitazone	30 mg	6 mo	Improved	Improved steatosis, ballooning and fibrosis (with vitamin E)	Not evaluated
Shadid et al. (2003) (535)	5	Open label	Pioglitazone	30 mg	4–5 mo	Improved	Not evaluated	Not evaluated
Neuschwander-Tetri (2003) (536)	30	Open label	Rosiglitazone	8 mg	48 wk	Improved	Improved steatosis, inflammation, and fibrosis	CT scan: Improved
Promrat et al. (2004) (296)	18	Open label	Pioglitazone	30 mg	48 wk	Improved	Improved steatosis, inflammation, and fibrosis	MRI: Improved

Tiikkainen et al. (2004) (537)	9	Open label	Rosiglitazone	8 mg	16 wk	Improved	Not evaluated	MRI: Improved
METFORMIN								
Coyle et al. (1999) (538)	2	Open label	Metformin	500 mg	4 mo	Improved	Improved inflammation	Not evaluated
Marchesini et al. (2001) (539)	14	Open label	Metformin	1.5 g	4 mo	Improved	Not evaluated	U/S: Decreased hepatomegaly
Lavine et al. (2004) (540)	10	Open label	Metformin	1 g	6 mo	Improved	Not evaluated	MRI: Improved
Nair et al. (2004) (541)	15	Open label	Metformin	20 mg/kg	12 mo	Improved	Improved inflammation	Not evaluated
Uygun et al. (2004) (542)	36	Open label	Metformin	1.5 g	6 mo	Improved	Improved inflammation	U/S: Improved
Tiikkainen et al. (2004) (537)	11	Open label	Metformin	2 g	16 wk	Improved	Not evaluated	MRI: No improvement
Buigianesi et al. (2005) (543)	55	Open label	Metformin	2 g	12 mo	Improved	Improved steatosis, inflammation, and fibrosis in limited sample	Not evaluated
CYTOPROTECTIVE								
Guma (1997) (544)	24	RCT	UDCA	10 mg/kg	6 mo	Improved	Not evaluated	Not evaluated
Ceriani (1998) (545)	31	RCT	UDCA	10 mg/kg	6 mo	Improved	Not evaluated	Not evaluated
Laurin (2002) (546)	24	Open label	UDCA	13–15 mg/kg	12 mo	Improved	Improved steatosis	Not evaluated
Mendez-Sanchez (2002) (547)	33	RCT	UDCA	1,200 mg	6 wk	Improved	Not evaluated	Not evaluated
Santos (2003) (548)	30	RCT	UDCA	10 mg/kg	3 mo	Improved	Not evaluated	Not evaluated
Bauditz (2004) (549)	12	Open label	UDCA	7–10 mg/kg	6 mo	Improved	Not evaluated	Not evaluated
Lindor (2004) (550)	166	RCT	UDCA	13–15 mg/kg	24 mo	Improved	Improved steatosis	Not evaluated
VITAMIN E								
Lavine (2000) (551)	11	Open label	Vitamin E	400–1,200 IU	4–10 mo	Improved	Not evaluated	Not evaluated
Hasegawa (2001) (552)	12	Open label	Vitamin E	300 mg	12 mo	Improved	Improved steatosis, inflammation, and fibrosis	Not evaluated
Sanyal (2004) (534)	10	Open label	Vitamin E	400 IU	6 mo	Improved	Mild improved steatosis	Not evaluated

Harrison (2003) (553)	45	RCT	Vitamin E	1,000 IU	6 mo	Improved	Improved fibrosis (with vitamin C)	Not evaluated
Kugelmas (2003) (554)	16	RCT	Vitamin E	800 IU	3 mo	Improved	Not evaluated	Not evaluated
Vajro (2004) (555)	28	RCT	Vitamin E	400–1,000 IU	5 mo	Improved	Not evaluated	Not evaluated
Kawanaka (2004) (556)	10	Open label	Vitamin E	300 mg	6 mo	Improved	Not evaluated	Not evaluated

RCT, randomized controlled trial; CT, computerized tomography; MRI, magnetic resonance imaging; U/S, ultrasonography; UDCA, ursodeoxycholic acid.

Dietary Weight Loss and Exercise

Diet- and exercise-induced weight loss has shown promise in treating NAFLD but may also be associated with progression of liver disease if the rate of loss is greater than 1.6 kg/week and especially with drastic calorie reduction (306,573,574,575,576,577). In addition, weight loss may not restore normal insulin secretory pulses, suggesting persistent islet cell dysfunction in some patients (578). However, improvement in liver enzymes and histology has been shown with nutritional counselling and weight reduction (579,580). Ueno et al. reported significant improvement in liver enzyme levels and degree of steatosis in 15 obese patients treated with diet (25 kcal/kg ideal body weight per day) and exercise (walking and jogging) for 3 months compared to a control group, but fibrosis was not significantly altered (581). Not surprisingly, clinical improvement has been correlated with improving insulin signaling (582). Nonetheless, the paucity of more definitive data on this practical form of intervention is striking (583).

Weight Loss in Children

A number of small studies support the use of weight loss therapy in children. In one study, 33 obese children underwent a moderate hypocaloric diet (35 kcal/kg per day) and aerobic activities (6 hour/week or more) to achieve weight loss of approximately 500 g/week over a period of 6 months (584). All patients who lost at least 10% body weight had normalized liver enzyme levels and improved ultrasonographic findings. In a similar study, seven patients were treated with hypocaloric diet and exercise to obtain a weight loss approximately 500 g/week. All had significant reduction of aminotransferase levels; four out of five patients who had previous ultrasonography showed decreased evidence of steatosis. One patient who had a follow-up liver biopsy showed improvement in steatohepatitis (585).

Dietary Composition

Dietary lipid composition may be especially important for both insulin signaling and risk of lipid peroxidation (586). Dietary fat predominantly (98%) consists of triglyceride (glycerol and three fatty acids). Fatty acids affect the phospholipid composition of cell and organelle membranes and influence insulin sensitivity, gene regulation (PPARs), differentiation in adipocytes, and prostaglandin physiology (361,587,588,589). Fatty acids reach the liver from albumin-bound fatty acids released by adipose tissue and from chylomicron remnants directly from dietary fat (361,590). Diets enriched in PUFA (fish oil or ω-3 fatty acids) have not been adequately investigated for their potentially beneficial or detrimental effects in NAFLD, but some encouraging data (see subsequent text) has begun to emerge in spite of concerns for the role of ω-3 fatty acids in alcohol-related liver disease (591,592,593).

A study in MCD mice demonstrated that hepatic fatty acid content influences PPARα activity, which subsequently influences the activity of cytochrome P-450; this appears to promote lipid turnover in vivo and improves steatohepatitis (594). Supplementation of the diet with ω-3 fatty acids reduced collagen content in mice exposed to thioacetamide, whereas a mixture of ω-3 and ω-6 fatty acids had no effect (595). Lipid composition has also been shown to influence the expression of UCP-2 in the rat liver (596), and supplementing of ω-3 fatty acids in both the ob/ob mouse model and in the Fisher 344 leptin-resistant rat increased fat degradation factors (PPARα) and decreased hepatic fat synthetic factors (SREBP-1) (597,598). Conjugated dienoic derivatives of linoleic acid (conjugated linoleic acid [CLA]), have variable effects on hepatic and peripheral fat stores, depending on the content of other dietary fats (599). In humans, ongoing trials using indirect indices of NASH (e.g., aminotransferases, echotexture, Doppler blood flow, TNF) indicate improvement with ω-3 supplements (e.g., eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) (600,601,602).

Weight Loss Supplements

Orlistat (tetrahydrolipostatin) decreases fat absorption by inhibiting lipase. Harrison et al. have reported benefit in weight loss (mean of 10 kg) and histologic parameters (i.e., steatosis and fibrosis) in two pilot studies (603,604). Other existing studies are of relatively short duration and uncontrolled but have shown general improvement in surrogate markers of injury (605). Malabsorption of fat-soluble vitamins (including vitamin E) is of theoretic concern. There is presently too little data on sibutramine (a serotonin and norepinephrine reuptake inhibitor that is approved as a weight loss agent), although one uncontrolled study

has suggested improved aminotransferases with associated weight loss (575,605).

Weight-Reduction Surgery

Variations of gastric bypass or gastric restriction are becoming increasingly popular, although these interventions are not without the risk of hepatic decompensation (606,607). Early studies showed a reduction in the levels of markers of metabolic syndrome (e.g., glucose, insulin, fibrinogen, triglycerides, and uric acid) and ALT levels and a reduction in steatosis but a slight overall increase in inflammation and no significant change in fibrosis. However, reduced perisinusoidal fibrosis was reported in another study by Silverman et al. (99). Moreover, resolution of histologic NASH was observed in one study using adjustable gastric banding, and an even more substantial reduction in fibrosis was recently reported in one large series, although caution is warranted, given the potential for deterioration with too rapid weight loss (608,609,610). Prebypass treatment with ursodeoxycholic acid (UDCA) and vitamin E has been advocated in patients with active NASH but has not been studied in a controlled trial.

Ursodeoxycholic Acid and Cytoprotective Agents

The potential benefits of UDCA derive from its effects on mitochondrial membrane stability, improved blood flow, and/or immunomodulation (611,612,613,614,615,616). Several studies, some published only as abstracts, suggested a potential benefit of

UDCA (544,545,546,547,548,549). In a fully published paper, Laurin treated 24 patients with NASH using UDCA (13 to 15 mg/kg) and observed improvement in liver enzyme levels and amelioration of steatosis (without change in fibrosis or inflammation) on biopsy at 12 months. Similarly, Ceriani et al. demonstrated normalization of liver enzymes in 14 of 16 patients treated with UDCA (10 mg/kg) compared to 4 of 15 on placebo and Guma et al. demonstrated liver enzyme normalization in 10 of 13 treated patients (10 mg/kg per day) versus 3 of 11 patients in a placebo group. However, in the largest study, Lindor et al. conducted a randomized trial in 166 patients (550). All patients were encouraged to lose weight and randomized to UDCA (13 to 15 mg/kg per day) or placebo for 2 years. Both groups had similar improvement in aminotransferases, and follow-up biopsy, available in two thirds of the patients, revealed similar levels of improvement in both groups. Whether negative results from this controlled trial reflect a true lack of response from UDCA is debatable (617). The surprising improvement in the placebo group in this study has pointed out the need for controlling for voluntary lifestyle changes when assessing pharmacologic intervention. Another recent randomized controlled trial of 48 patients (available only as abstract), in which patients were treated with the combination of UDCA (12 to 15 mg/kg) and vitamin E (800 IU/day) for 2 years versus single or double placebo controls, showed significant improvement in NAS only in the group treated with combination therapy (618).

Other agents with potential cytoprotective properties include taurine, an amino acid that alters bile acid physiology and that has been reported to normalize liver enzymes in children with NAFLD (619). Triacetyl uridine is another potentially important cytoprotective agent in experimental choline-deficient rats but has not been tested in humans (620).

Antioxidants and Nutritional Supplements

Vitamin E supplementation has been studied in both experimental and clinical settings (621). "Vitamin E" refers to a family of tocopherols and tocotrienols (each with different forms named α, β, γ, and δ) that exhibit antioxidant activity. Commercially available vitamin E supplements usually contain only α-tocopherol—the relative efficacy of mixed tocopherols compared to α-tocopherol alone is not established (622,623,624,625). Experimentally, a number of encouraging studies have been published (626,627). Similarly, human studies have shown reduced lipid peroxidation and cytokine levels with vitamin E therapy (552,556). However, pilot studies and two randomized controlled studies have produced varying results (551,553,554,555). A placebo-controlled trial in 14 children showed liver enzyme improvement with vitamin E (400 IU daily for 2 months followed by 100 IU daily for 3 months), but the placebo group also demonstrated a significant improvement in liver test results in parallel with weight loss. In adults, Harrison et al. randomized 45 patients with biopsy-proven NASH to vitamin E (1,000 IU daily) plus vitamin C (1,000 mg daily) versus placebo. Repeat liver biopsy after 6 months showed a small improvement in fibrosis score in the vitamin E group; however, there was no significant improvement in inflammation or necrosis score on biopsy. A second controlled study in 16 adults showed no benefit of vitamin E over diet and exercise alone. Although caution is warranted in patients with prior coronary artery disease where vitamin E is associated with blunted efficacy of statin drugs (628), significant toxicity has not been observed in these studies, but concerns over doses exceeding 400 IU/day (629) and the limited efficacy have tempered enthusiasm (630). On the other hand, its use in combination with other agents (e.g., UDCA and pioglitazone) has been more encouraging.

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Betaine (trimethylglycine), a methyl donor in an alternative pathway for remethylation of homocysteine to methionine and *S*-adenosyl-methionine (SAMe), promotes the conversion of phosphotidylethanolamine to phosphotidylcholine (lecithin), which, in turn, promotes the export of fat from the liver as VLDL (631). Substantial benefit has been reported by Abdelmalek et al. in ten patients with NASH treated with betaine solution (20 g/day for 10 to 12 months) in terms of biochemical and multiple histologic parameters including steatosis, inflammation, and fibrosis (632). These agents may also serve to replenish glutathione and improve mitochondrial membrane fluidity (633,634,635). Silymarin, the active component of milk thistle extract, is an extraordinarily popular over-the-counter supplement that has been observed to decrease the expression of CYP 3A4 and decrease mitochondrial respiration in hepatocyte culture but has not, to our knowledge, been carefully studied in NASH (636). *N*-Acetylcysteine, which is converted to glutathione in the liver, has produced improvement in liver enzymes (histology not done) in a small pilot study at a dosage of 1 g/day for 3 months (637). Lecithin increases plasma free choline and decreases hepatic steatosis in long-term TPN patients (638,639). Experimentally, probiotics have shown some encouraging results, possibly through their effects on secondary mediators of inflammation (640). Lazaroids, or 21-aminosteroids, are additional antioxidants that have not been studied in NASH but warrant consideration (641).

Antidiabetic Agents

Because insulin resistance appears to be the crucial problem in most patients with NASH, insulin-sensitizing agents have emerged as the most promising form of pharmacologic therapy, especially in those with progressive fibrosis (Figs. 39.15, 39.16 and 39.17) (642). Other antidiabetic strategies agents have been less well studied (643). For example, neither insulin nor sulfonylureas have been carefully studied for effects on fatty liver. The major adverse effects of insulin is weight gain with truncal obesity that could exacerbate NAFLD (644). On the other hand, control of blood sugar level in patients with overt diabetes will likely decrease de novo fat synthesis in the liver through effects on CREBP. Acipimox is an inhibitor of lipolysis that is thought to improve insulin sensitivity by lowering free fatty acid levels but its effect on NAFLD has not been studied (645). Similarly, experimental antidiabetic agents such as D-chiro-inositol and newer agents such as the incretins have not, to our knowledge, been studied in NAFLD (646,647).

Thiazolidinediones

TZDs are a group of agents that act as ligands for the peroxisome-proliferator–activated receptor-γ (PPAR-γ) (expressed in adipose tissue, intestines, macrophages, and muscle and activated by fatty acids),

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which is a member of a nuclear receptor superfamily that regulates gene expression of enzymes involved in lipid and glucose metabolism. The PPAR-γ receptor, the ligand, and a coactivator form a heterodimer with retinoid X receptor, which binds to the PPAR-γ response element of specific genes (648). The most profound effect of TZDs is in adipocyte differentiation (649). As a result, TZDs are often associated with increased peripheral but decreased central adiposity (650,651). Other effects include increased expression of glucose transporters (652), increased mitochondrial mass (653) and altered thermogenesis (through effects on UCP) (654), decreased cytokine expression, and inhibited inducible nitric oxide synthase, as well as decreased ceramide-induced apoptosis in pancreatic islet cells (655,656). Because PPAR-γ receptors are not

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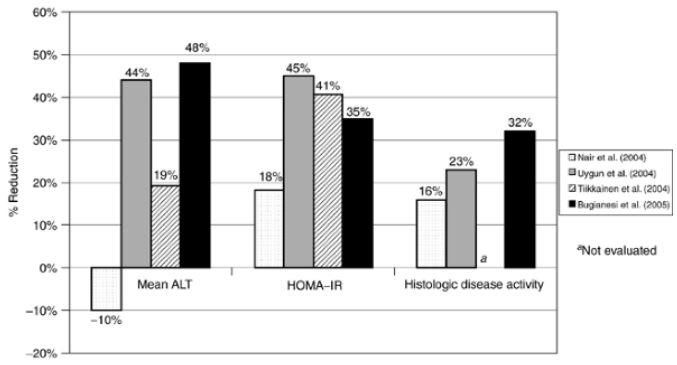
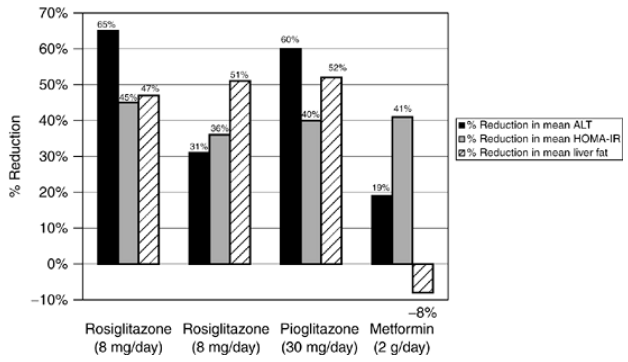
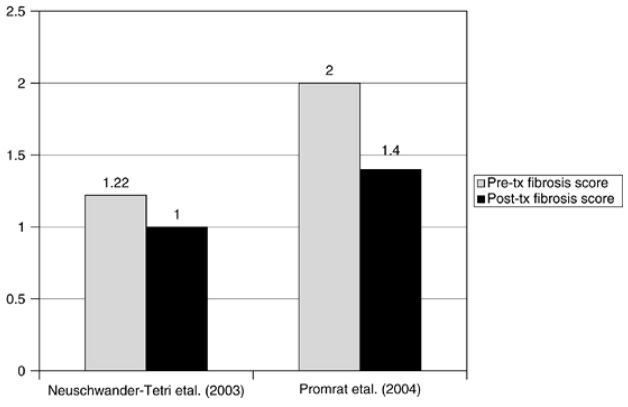
highly expressed in liver, the benefit to patients with NASH is likely to primarily involve indirect mechanisms.



• **Figure 39.15** Improvement in insulin resistance and histology with metformin in patients with nonalcoholic steatohepatitis. Since 1999, several studies have demonstrated metformin's beneficial effects on biochemical, histologic, and metabolic parameters associated with NASH. The histologic parameter was standardized across all four studies by calculating the nonalcoholic fatty liver disease activity score (NAS) from data presented in the studies. ALT, alanine transaminase. (Adapted from references 537,541,542,543.)



• **Figure 39.16** Comparison of thiazolidinediones (TZDs) with metformin shows greater improvement in biochemical, metabolic, and hepatic steatosis in the former. Equivalent doses of TZDs show similar effects on alanine transaminase (ALT), insulin resistance, and hepatic steatosis, which are thought to be due to their effects on mobilization of central fat to peripheral areas. Although these data are not conclusive, metformin appears to have a more modest effect compared with the TZDs. (Adapted from references 296, 536, 537.)



• **Figure 39.17** Thiazolidinediones appear to improve fibrosis after treatment. In the studies by Neuschwander-Tetri et al. and Promrat et al., fibrosis scores on posttreatment (tx) liver biopsies had significantly decreased after 6 months of treatment with either rosiglitazone or pioglitazone. (Adapted from Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, et al. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-γ ligand rosiglitazone. *Hepatology* 2003;38:1008–1017.and Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004;39:188–196.)

A 4- to 6-month course of troglitazone normalized liver enzymes in seven of ten patients with NASH and improved inflammation in four of seven with follow-up biopsies (most with loss of polymorphonuclear cells) with no change in fibrosis scores (213). Long-term histologic follow-up 4 to 5 years after completing the study in these patients has shown near resolution in one patient who had subsequent success with weight loss and diet and progression to uncomplicated cirrhosis in another patient who was unable to achieve

lifestyle changes (657). Rosiglitazone has been evaluated in two publications by Neuschwander-Tetri et al. (536,658). These studies showed significant improvement in the posttreatment biopsy and almost one half of the patients no longer met the histologic criteria for NASH. These changes were paralleled by improvement in indices of insulin activity and decreased hepatic fat content. Consistent with the effect of TZD on peripheral fat stores, 67% gained weight during the study (median increase of 7.3%). Laboratory follow-up on this group of patients also showed a relapse of the aminotransferase levels to baseline levels, indicating that the changes obtained were not sustained.

A similar effect of pioglitazone in both aminotransferase levels and histologic parameters has also been observed (533,535,659). Sanyal et al. performed a controlled trial of pioglitazone versus vitamin E, showing superiority of TZD in terms of histologic remission (534). The most detailed report available is that by Promrat et al. who treated 18 patients with pioglitazone, 30 mg daily, for 48 weeks with endpoints of histologic activity, insulin activity, body composition (dual-energy x-ray absorptiometry), and hepatic fat content by MRI (296). All patients received recommendations for increased physical activity, reduced calorie diet, and a daily multivitamin. Therapy was associated with normalization of the aminotransferase levels, a significant reduction in a composite histologic score (the NAI—see preceding text), a reduction in steatosis confirmed by MRI, and improved insulin sensitivity. Sustained weight gain was seen in 72% of patients (average of 3.5 kg). Preliminary results from a placebo-controlled trial indicate a correlation between higher adiponectin levels, reduced steatosis, and improved fibrosis in the treated group (660).

In the existing pilot studies (including troglitazone), elevation of liver enzyme levels during treatment has been reported in approximately 3 of 79 patients (one each for the major TZDs studied). No cases of severe hepatitis or exacerbation of NASH has been noted. Although troglitazone was well tolerated even in the presence of stable cirrhosis, a rare but potentially severe idiopathic toxicity led to its removal from the market (661,662,663). Even more rare toxicity has been reported with rosiglitazone and pioglitazone (664,665,666). The mechanism of idiosyncratic toxicity with TZDs is uncertain, but several lines of evidence (including a combined toxicity of troglitazone with an HMG-CoA reductase inhibitor) (667) point to rare but potentially significant mitochondrial dysfunction. Weight gain is the most common side effect noted in the existing studies. It is suspected that successful dietary and exercise intervention can prevent this development, but predictive factors remain to be defined. The effect of shifting fat stores on myocyte function, exercise tolerance, and endurance in these patients has not been adequately addressed. Edema, sometimes associated with congestive heart failure, has been reported, but the relationship between the medication and cardiac dysfunction is debated (668,669).

Metformin

Metformin is an insulin-sensitizing, oral biguanide approved for use in type 2 diabetes in the United States since 1995 (670). The biguanides exert substantial changes in cellular bioenergetics without inducing weight gain, by reducing hepatic glucose production and increasing peripheral glucose utilization (671). Its major site of action is the mitochondria and causes a reduction of ATP in isolated rat hepatocytes, reduced activity of complex I in the mitochondrial respiratory chain, reduced fatty acid oxidation, and increased lactate production in preadipocytes under experimental conditions (653,672,673). Signaling is through adenosine monophosphate–activated protein kinase—this pathway is similarly activated with exercise, suggesting that in some ways metformin simulates the effects of exercise on glucose transport (674).

Favorable studies of metformin in the fatty liver of ob/ob mouse (675) along with a favorable side effect profile, especially the absence of weight gain seen with the TZDs, have led to sustained interest in this agent in treating NASH. Coyle et al. noted that metformin improved overall histology in two patients in one of the first reports of histologic changes (538). Less favorably, Tiikkainen et al. in a human study in patients with type 2 diabetes examining the relative effects of metformin (2 g/day) and rosiglitazone (8 mg/day), for 16 weeks, on steatosis and serum adiponectin levels, revealed that both drugs similarly increased insulin sensitivity but only rosiglitazone reduced hepatic steatosis (537). However, Marchesini et al. demonstrated improved liver enzymes, reduced hepatomegaly, and improved hepatic perfusion in patients treated with metformin (1,500 mg/day) for up to 6 months, and in a follow-up study, Bugianesi et al. reported improvement in several histologic parameters (e.g., steatosis, necroinflammation and fibrosis) independent of dietary changes (539,543,676).

Lavine and Schwimmer reported similar histologic improvement in ten children along with reduced steatosis by MRS (540). However, less definitive improvement in histologic parameters were observed in two other studies by Uygun et al. and Nair et al. (541). The overall safety profile of metformin is favorable and lactic acidosis appears to be rare, but caution is warranted in renal insufficiency (677).

Antihyperlipidemic Agents

Fibrates are lipid-lowering agents that act on PPAR-α receptors located primarily in the liver, heart, muscle, and kidney (normally activated by adipose tissue–derived fatty acids) that increase fatty acid uptake, mitochondrial β-oxidation, peroxisomal oxidation of fatty acids, and ω-oxidation of fatty acids in the P-450 system (648). However, histology-based studies of fibrates have not shown significant beneficial effects, although the available data is limited and some studies have reported improved transaminase levels (546,678,679).

With statin drugs (HMG-CoA reductase inhibitors), some type of interaction with NAFLD seems likely because the drugs are acting in part on hepatic fat metabolism and two thirds to three fourths (noninvasive testing) of hyperlipidemic patients have NAFLD. Not much is known about the long-term effects of these agents on NAFLD; however, the incidence of acute hepatitis, judged by the aminotransferase levels, appears to be quite low (108,680). On the other hand, induction of SREBP (a transcription factor governing hepatic fat synthesis), diminished secretion of VLDL and perhaps depleted ubiquinone raise concern about long-term effects (106). The anti-inflammatory effects of the statins may cause decreased cellular injury although there is reason to suspect that these agents will increase liver fat stores. However, limited available studies suggest an overall beneficial effect in NAFLD (107,601,681,682). It seems likely that this complex interaction represents the interaction between a number of covariables including the degree of physical conditioning (insulin signaling), ethanol use, dietary fatty acid composition, and genetic variables in fatty acid metabolism. Additional data is needed, especially in light of efforts to make these agents available "over-the-counter" (683).

Other Agents

Recent experimental data from Diehl et al. indicate that modulation of the adrenergic system may significantly influence NAFLD (370,684). A close association between the sympathetic nervous system (SNS) and the fatty liver seems likely on the basis of the close association between obesity and the SNS (685). Whether pharmacologic manipulation of the system in humans might be beneficial in select cases remains to be investigated. Other agents, also related more classically to the cardiovascular system, include angiotensin receptor blockers (ARBs). One small uncontrolled study in human patients with NASH and hypertension treated with losartan has shown histologic benefit after 48 weeks (686).

Liver Transplantation, Disease Recurrence, and Donor Organs

Transplantation for patients with advanced NASH is often complicated because of the presence of comorbid conditions related to obesity, diabetes, and hyperlipidemia (687,688). Although guidelines do not exist, these conditions warrant careful consideration of the long-term benefits of transplantation in this setting. Recurrence of disease is an additional concern. A number of reports have now documented the recurrence of NAFLD and NASH after transplantation (689,690,691,692). In addition, two series have shown a high risk for the development of NAFLD in patients with cryptogenic cirrhosis after liver transplantation (see "Cryptogenic Cirrhosis"). Immunosuppression regimens likely play a role: Steroid therapy promotes fatty change and cyclosporine interacts with the mitochondrial transition pore that regulates the electrochemical gradient across the mitochondrial membrane (693). The relative risks and benefits of different immunosuppression regimens in these patients are not established but the course may be severe. Steatosis in donor livers is associated

with poor graft function likely due to abnormal mitochondrial function and disturbed ATP homeostasis (see "Pathogenesis of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis") (694). The problem has become magnified with efforts to develop living-related donor programs in which prediction of steatosis in a partial donor can be difficult (695). The role of preoperative dietary changes in prospective living donors remains to be fully investigated (696).

Annotated References

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Dixon JB, Bathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001;121:91–100.

Garcia-Monzon C, Martin-Perez E, Lo Iacono O, et al. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. *J Hepatol* 2000;33:716–724.

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Ratzui V, Bonyhay L, Di Martino V, et al. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 2002;35:1485–1493.

Ratziu V, Giral P, Charlotte F, et al. Liver fibrosis in overweight patients. *Gastroenterology* 2000;118:1117–1123.

These papers have provided important observations about the prediction of severity of underlying liver disease in consecutive obese patients or, in the Angulo paper, among consecutive patients with NASH. Among the salient findings were associations of more severe histology with increased obesity, more severe insulin resistance, hypertension, hypertriglyceridemia, elevation of transaminases (not invariably present) and/or AST:ALT ratio more than 1, age over 40 to 50 years, and female gender. From serial biopsy studies in patients with NASH, cirrhosis develops in about 10% while fibrosis progresses in 33%, remains stable in 41%, and improves in 22% over about 5 years. Once cirrhosis develops, there is a significant risk of decompensated portal hypertension and hepatocellular carcinoma.

Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histologic lesions. *Am J Gastroenterol* 1999;94:2467–2474.

Kleiner DE, Brunt EM, Van Natta ML, et al. Nonalcoholic steatohepatitis clinical research network. Design and validation of a histologic scoring system for NAFLD. *Hepatology* 2005;41:1313–1321.

Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116:1413–1419.

The paper by Matteoni et al. elucidated the relationships between types of NAFLD, including simple steatosis or steatosis with mild inflammation (types 1 and 2), and more severe histology associated with fibrosis (types 3 and 4). Types 3 and 4 are associated with greater likelihood of progression. The paper by Brunt et al. provided a widely referenced scoring scheme for grading and staging NASH. Many of the key histologic parameters have subsequently been combined by Kleiner et al. into a composite score (the NASH activity score or NAS) that will facilitate assessment of therapeutic interventions and comparison between different treatments.

Caldwell SH, Oelsner DH, Iezzoni JC, et al. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999;29:664–669.

Contos MJ, Cales W, Sterling RK, et al. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. *Liver Transpl* 2001;7:363–373.

Ong J, Younossi ZM, Reddy V, et al. Cryptogenic cirrhosis and post-transplantation non-alcoholic fatty liver disease. *Liver Transpl* 2001;7:797–801.

Powell EE, Cooksley WG, Hanson R, et al. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990;11:74–80.

The paper by Powell et al. demonstrated a key observation that NASH may progress to a "bland" cirrhosis with loss of histologic features. This relationship was extended by Caldwell et al. to show that many cases of cryptogenic cirrhosis likely result from prior, unrecognized NASH in obese patients—especially women. An age difference of approximately 10 years (mean age 50 in NASH and 60 in cirrhosis) suggests that the sixth decade is commonly associated with progression to cirrhosis in patients with NASH. The high occurrence (recurrence) of NASH after liver transplantation shown in studies by Contos et al. and Ong et al. indicates the potential severity of the disease and further strengthened the suspected relationship between NASH and cryptogenic cirrhosis.

Cortez-Pinto H, Chatham J, Chacko VP, et al. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* 1999;282:1659–1664.

Day CP, James OFW. Steatohepatitis: a tale of two hits. *Gastroenterology* 1998;114:842–845.

Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005;115:1343–1351.

Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathway. *Hepatology* 2004;40:185–194.

Hui JM, Hodge A, Farrell GC, et al. Beyond insulin resistance in NASH: TNF-α or adiponectin? *Hepatology* 2004;40:46–54.

Pessayre D, Fromenty B. NASH: a mitochondrial disease. *J Hepatol* 2005;42:928–940.

Accumulation of hepatic lipid, the first step in the "two-hit" hypothesis (steatosis and oxidative stress) of NASH results from both increased uptake of fatty acids from the periphery and from de novo lipogenesis within the liver. Resulting lipid peroxidation underlies many of the changes that ultimately develop into progressive NASH including mitochondrial dysfunction (in both liver and other organs), activation of apoptosis pathways, cytokine synthesis, and activation of stellate cells with resulting fibrosis. The "communications," through cytokines and adipocytokines, between fat stores and targets of insulin activity appear to play a significant role in the ultimate stability of the problem or the development of cellular injury.

Huang MA, Greenson JK, Chao C, et al. One-year intense nutritional counseling results in histological improvement in patients with nonalcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 2005;100:1072–1081.

Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, et al. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-γ ligand rosiglitazone. *Hepatology* 2003;38:1008–1017.

Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004;39:188–196.

Ratziu V, Charlotte F, Heurtier A, et al. LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005;125:1898–1906.

Although there is yet surprisingly limited data, diet and exercise, most likely through their effect on insulin signaling and energy regulation, are the cornerstones of therapy, as demonstrated in the report by Huang et al. These voluntary lifestyle changes (diet and exercise) and sampling variability on liver biopsy noted in the study by Ratziu et al. are two of the most significant confounding variables that must be considered in assessing clinical trials of pharmacologic therapy (aimed especially at patients with evidence of ongoing histological injury). Using a variety of clinical endpoints in addition to histology—such as global measures of hepatic steatosis, anthropometric measures, insulin signaling, and cytokine levels—the insulin-sensitizing agents known as thiazolidinediones (e.g., rosiglitazone and pioglitazone) are emerging as the most promising agents, although side effects and durability of the response are yet to be fully defined in controlled trials.

Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999;107:450–455.

Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001;120:1183–1192.

Using different methods to assess insulin activity, these two papers demonstrated insulin resistance as a key feature of NAFLD. Salient observations including demonstration of insulin resistance in obese and lean patients with fatty liver, shown by Marchesini et al. and extensive analysis of fat metabolism in NASH, by Sanyal et al. indicated diminished

suppression of lipolysis with insulin infusion and increased rates of fatty acid oxidation associated with oxidative stress and mitochondrial injury in NASH.

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Chapter 40
Vascular Diseases of the Liver

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Key Concepts

- Budd-Chiari syndrome occurs as a consequence of thrombotic occlusion of hepatic venous outflow.
- Hypercoagulable states account for most cases of Budd-Chiari syndrome. Anticoagulation is the mainstay of medical management.
- Webs and membranes in the inferior vena cava develop from prior thrombosis and represent the most common subtype of Budd-Chiari syndrome in the Far East.
- Acute or subacute Budd-Chiari syndrome may present with abdominal pain, hepatomegaly, and ascites, whereas symptoms and signs of portal hypertension are prominent with chronic disease.
- Doppler ultrasonography is a useful noninvasive screening test for Budd-Chiari syndrome. Contrast-enhanced computed tomography and magnetic resonance imaging can also demonstrate hepatic vein occlusion and associated parenchymal abnormalities secondary to venous outflow obstruction. Venography and liver biopsy remain the gold standard for diagnosis.
- Angioplasty and thrombolysis may be of benefit in highly selected cases. Decompressive surgery and transjugular intrahepatic portosystemic shunt are often reserved for progressive liver disease despite medical management. Liver transplantation may be performed for patients with decompensated cirrhosis or acute liver failure.
- Hereditary hemorrhagic telangiectasia, also known as *Rendu-Osler-Weber syndrome*, is a multisystemic vascular disorder that may involve the liver.
- Peliosis hepatis is characterized by blood-filled cystic lesions in the hepatic parenchyma, which may occur in the setting of immunosuppression or human immunodeficiency virus (HIV) infection. *Bartonella henselae* is the cause of bacillary peliosis hepatis in patients with HIV infection.

Budd first described a clinical triad of abdominal pain, ascites, and hepatomegaly in 1845 (1). Chiari later provided pathologic correlation (2). Today, Budd-Chiari syndrome refers to the thrombotic obstruction of the hepatic venous outflow system. Occlusion may occur in hepatic venules, hepatic veins, and the inferior

vena cava up to the right atrium. Budd-Chiari syndrome must be distinguished from other causes of hepatic venous outflow obstruction, including right heart failure and veno-occlusive disease. In the Far East, occlusion of the inferior vena cava with webs and membranes is more common, whereas in the West, hepatic vein thrombosis is typical. Hypercoagulable states are responsible for most cases of Budd-Chiari syndrome, and anticoagulation is the mainstay of medical treatment. Clinical presentation is variable and may depend on the extent and speed of occlusion, as well as the development of collateral circulation. Acute, subacute,

chronic, and asymptomatic presentations have all been well described. Hepatic venography is the diagnostic standard and is of value when contemplating surgical or percutaneous shunting. In the absence of rigorous, prospective studies, treatment algorithms are difficult to define. Angioplasty and thrombolysis may be used in highly selected patients. Transjugular intrahepatic portosystemic shunt (TIPS) and surgical decompression are options for progressive liver disease despite anticoagulation and other medical therapy. Patients with decompensated cirrhosis or acute liver failure may require liver transplantation.

Hereditary hemorrhagic telangiectasia (HHT), also known as *Rendu-Osler-Weber syndrome*, is characterized by presence of epistaxis, mucocutaneous telangiectasias, and visceral arteriovenous malformations. Hepatic involvement is variably present but is usually asymptomatic. Hepatic arterial embolization has been used for symptomatic disease, but recent experience highlights the dangers of this approach.

Peliosis hepatis is characterized by cystic, blood-filled lesions in the liver. It occurs in patients who are immunosuppressed or have human immunodeficiency virus (HIV) infection. *Bartonella henselae* is the causative agent of bacillary peliosis hepatis associated with HIV. Treatment of these generally asymptomatic lesions consists of either administering antibiotics to treat *Bartonella* infection or addressing the underlying cause of immunosuppression.

Budd-Chiari Syndrome and Related Disorders

Etiology

The most frequent causes of venous obstruction are prothrombotic disorders, particularly myeloproliferative diseases (3,4,5) (Table 40.1). In one of the largest series, 45% of patients with Budd-Chiari syndrome had polycythemia rubra vera and 9% were diagnosed with essential thrombocythemia (6). Occult myeloproliferative states, demonstrated by spontaneous endogenous erythroid colony formation in the absence of erythropoietin stimulation, are also common (7). An increasing number of thrombotic disorders have been associated with Budd-Chiari syndrome (8,9,10,11,12,13). In over 25% of cases, more than one thrombophilic state may be present (13). Careful, systematic evaluation for prothrombotic disorders has lowered the proportion of cases labeled as idiopathic to less than 10% (7,13,14). Factor V Leiden mutation is the most frequent cause of hereditary thrombophilia and is thought to be the second most common cause of thrombotic occlusion of the hepatic veins and vena cava (8,9,10,11,12,13,14,15,16,17,18). Other hypercoagulable states associated with Budd-Chiari syndrome include antiphospholipid antibody syndrome, paroxysmal nocturnal hemoglobinuria, prothrombin G mutation, methylene-tetrahydrofolate

reductase mutation, antithrombin III deficiency, and deficiencies of protein C and protein S (13,15,19,20,21,22,23,24). Patients who are pregnant or are taking oral contraceptives and develop Budd-Chiari syndrome usually have an underlying thrombophilic state (8,9,10,11,12,13,14,15,16,17,18).

Table 40.1. Etiology of Budd-Chiari Syndrome

MYELOPROLIFERATIVE DISORDERS Polycythemia rubra vera Essential thrombocytosis Occult myeloproliferative disorders	**LOCAL COMPRESSION** Neoplasms Infection
OTHER HYPERCOAGULABLE STATES Factor V Leiden mutation Prothrombin G20210A gene mutation Antiphospholipid antibody syndrome Methylene-tetrahydrofolate reductase mutation Paroxysmal nocturnal hemoglobinuria Protein C and S deficiency Antithrombin III deficiency Oral contraceptives Pregnancy	**SYSTEMIC DISEASES** Behçet's disease Inflammatory bowel disease Sarcoidosis Idiopathic

Frequently more than one condition may be present.

Local compression by adjacent tumor, abscess, or inflammation may lead to a secondary Budd-Chiari syndrome. Neoplasms associated with outflow obstruction include primary hepatocellular, renal, adrenal, pulmonary, pancreatic, and gastric carcinomas (25,26,27,28,29). Benign and malignant vascular neoplasms arising within the hepatic veins or vena cava (e.g., cavernous hemangiomas, leiomyomas, leiomyosarcomas, and rhabdomyosarcomas) have also been associated with Budd-Chiari syndrome (30,31,32,33). Other rare causes of hepatic venous outflow obstruction have been identified. Bacterial, viral, and parasitic infections; collagen vascular diseases; inflammatory bowel disease; and Behçet's disease may result in venous occlusion (29,34,35,36,37,38,39,40,41,42).

Inferior vena cava thrombosis

Budd-Chiari syndrome includes thrombosis anywhere along the hepatic venous outflow tract. It has been proposed that obstruction principally affecting the inferior vena cava should be termed *obliterative hepatocavopathy* (43). Primary inferior vena cava thrombosis appears to be more common in India, China, Japan, Nepal, and South Africa for unclear reasons. Compared to classic

Budd-Chiari syndrome, obliterative hepatocavopathy is more often considered idiopathic (43,44,45,46,47). It has also been found in association with hepatocellular carcinoma (43,44,45,46,47). However, systematic investigation

frequently reveals an underlying thrombophilic state (44,48). The obstruction results from caval webs or membranes, which may also involve the ostia of the hepatic veins. Although the lesions were formerly thought to be congenital, it is now recognized that they represent the transformation of inferior vena cava thrombosis (43,49). Widespread acceptance of obliterative hepatocavopathy as a distinct entity has not occurred because its etiology, prognosis, and management are similar to those of classic Budd-Chiari syndrome (Table 40.2) (50).

Occlusion of the terminal hepatic venules (veno-occlusive disease)

Veno-occlusive disease refers to obstruction of the hepatic sinusoids or small intrahepatic veins. In contradistinction to Budd-Chiari syndrome, occlusion is not thrombotic but results from fibro-obliterative endophlebitis (Table 40.2). Occasionally, Budd-Chiari syndrome may involve only small intrahepatic veins. Sparing of the large hepatic veins can be seen with allergic phlebitis, granulomatous disease, paroxysmal nocturnal hemoglobinuria, and other thrombophilic states (13,50). Distinction from veno-occlusive disease can generally be accomplished by recognition of etiology and in some cases by demonstration of clot. Outside the setting of bone marrow transplantation, the two conditions may be indistinguishable.

Initial reports of veno-occlusive disease were attributed to ingestion of herbal teas containing large quantities of pyrrolizidine alkaloids (51,52). Today, veno-occlusive disease is seen almost exclusively in the setting of bone marrow transplantation, and the reader is referred to Chapter 60 for a detailed review. Hepatotoxic agents, particularly certain chemotherapeutic conditioning regimens, account for most cases (Table 40.2). Recipients of liver and renal allografts and patients exposed to azathioprine are also at risk for developing veno-occlusive lesions. Other rare conditions associated with veno-occlusive disease include vitamin A toxicity; arsenic poisoning; exposure to insecticide; administration of 6-thioguanine, intra-arterial 5-fluoro-2’-deoxyuridine, Thorotrast, and a combination of norethisterone with conditioning chemotherapeutic agents (51,53,54,55,56,57).

Table 40.2. Budd-Chiari Syndrome Subtypes and Veno-Occlusive Disease

CLASSIC BUDD-CHIARI SYNDROME
Thrombosis principally of hepatic veins
More common in western countries
Thrombophilic state most common etiology
OBLITERATIVE HEPATOCAVOPATHY
Webs and membranes (representing prior thrombosis) in inferior vena cava
More common in China, Nepal, India, Japan, and South Africa
Thrombophilic state, idiopathic and hepatocellular cancer common
VENO-OCCLUSIVE DISEASE
Nonthrombotic fibrosis of hepatic sinusoids and small intrahepatic veins
Almost exclusively seen in the setting of bone marrow transplantation
Mainly caused by conditioning chemotherapy

Pathology

Hepatic venous outflow obstruction is caused by thrombotic occlusion of the terminal hepatic venules, hepatic veins, or inferior vena cava. Generally, the disease is silent if only one hepatic vein is occluded. Obstruction may manifest as fibrous cord remnants of hepatic veins, short-length stenoses, membranes, webs, or occlusions of the hepatic venous ostia (19,43,49,58). In acute Budd-Chiari syndrome, the liver appears enlarged, smooth, and red purple because of congestion. In chronic disease, direct outflow from the caudate lobe to the inferior vena cava may compensate for venous outflow obstruction of major hepatic veins, resulting in caudate lobe hypertrophy with atrophy and cirrhosis of the remaining segments (59). In some cases caudate lobe hypertrophy may obstruct the intrahepatic portion of the inferior vena cava.

Histologic changes may be uneven and result in liver biopsy sampling errors. Centrilobular congestion and sinusoidal dilatation are seen with acute obstruction, whereas atrophy, necrosis, and centrizonal hepatocyte dropout with extension to periportal regions are associated with severe injury. Venous stasis and congestion lead to hypoxic damage and oxidative injury to hepatocytes (60,61). In chronic disease, there is complete obliteration of the central veins associated with centrilobular fibrosis that may culminate in cirrhosis (62,63,64). Periportal fibrosis may be more prominent if branches of the portal vein are concomitantly thrombosed because of stasis (65). Large, regenerative nodules are also commonly reported in areas exposed to a compensatory increase in arterial blood flow (60,65).

Clinical Presentation

About two thirds of patients with Budd-Chiari syndrome are women, with onset of symptoms usually in the late 30s (6,66). Clinical presentation is variable and depends on the extent and rate of outflow obstruction, as well as the development of collaterals (19,62) (Table 40.3). Presentation may range from

an asymptomatic state to fulminant hepatic failure, or to cirrhosis with

complications of portal hypertension (15,29,67,68). More than 85% of patients have hepatomegaly and ascites, whereas esophagogastric varices and splenomegaly may be seen in 40% to 60% of individuals (67). The presence of dilated subcutaneous veins over the body and trunk are more often associated with inferior vena cava obstruction (43,69). Acute obstruction is commonly accompanied by right upper quadrant abdominal pain, nausea, vomiting, hepatomegaly, and ascites (67). Jaundice and splenomegaly may be present with acute occlusion but are usually mild. Rarely, massive hepatocellular necrosis with acute hepatic failure may follow rapid and complete occlusion of all major hepatic veins (29,67). A subacute presentation of less than 6 months is characterized by vague right upper quadrant discomfort, hepatomegaly, mild-to-moderate ascites, and splenomegaly (67,68). Jaundice is either absent or mild. Chronic Budd-Chiari syndrome of greater than 6 months' duration generally presents with progressive ascites. It may also be accompanied by other complications of portal hypertension, such as bleeding varices, encephalopathy, coagulopathy, renal insufficiency, fatigue, and muscle wasting (19,29,62,67,68). Generally, cirrhosis is found only in patients with chronic disease. However, the demonstration of cirrhosis in biopsy specimens taken from patients with acute outflow tract obstruction provides argument that the current classification system leaves room for improvement (47,70).

Table 40.3. Clinical Manifestations of Budd-Chiari Syndrome

Right upper quadrant pain
Hepatomegaly
Ascites
Splenomegaly (rare)
Jaundice
Acute liver failure (rare)
Weight gain
Nausea and vomiting
Pleural effusions
Lower-extremity edema
Complications of portal hypertension

Diagnostic Evaluation

Laboratory investigation

Standard laboratory investigation is rarely helpful in patients with Budd-Chiari syndrome. Nonspecific mild transaminase level elevation can be seen in 25% to 50% of patients but does not aid in establishing the diagnosis (25,37). Transaminase values over 1,000 IU/L are possible in acute outflow obstruction or hepatic failure, especially if there is accompanying portal vein thrombosis (25). Serum bilirubin and alkaline phosphatase levels and prothrombin time are usually normal or mildly elevated and are also not specific (25,71,72). Ascitic fluid analysis is consistent with portal hypertension. Myeloproliferative and thrombotic disorders are common. Therefore, systematic, comprehensive evaluation for hypercoagulable disorders should be undertaken. The following tests should be performed: Plasma clotting factors and inhibitors, factor V Leiden factor

mutation, prothrombin G gene analysis, antiphospholipid antibodies and lupus anticoagulant, flow cytometry for paroxysmal nocturnal hemoglobinuria, blood smear analysis, and in select cases determination of total red cell mass, bone marrow biopsy, and measurement of serum erythropoietin levels (50,62). Caution must be exercised in the interpretation of protein C, protein S, and antithrombin III because compromised hepatic synthetic function may provide alternative explanations.

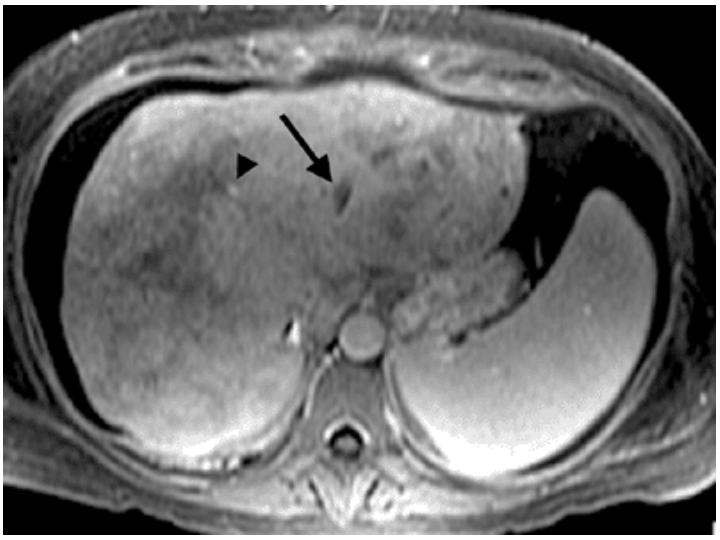
Medical imaging

Doppler ultrasonography allows for noninvasive evaluation of the hepatic veins, inferior vena cava, and portal vein. In experienced hands the sensitivity of ultrasonography for detecting venous obstruction approaches 85% to 95% (15,19,73). Real-time evaluation of acute venous occlusion reveals enlarged, stenotic, or tortuous hepatic veins, whereas the major hepatic veins of patients with chronic disease may not be seen (73,74,75,76). Intrahepatic venous-to-venous spider web collaterals and/or the presence of intrahepatic or subcapsular hepatic venous collaterals are highly suggestive of Budd-Chiari syndrome (50,73,74). Ultrasonography may also show caval compression by a hypertrophied caudate lobe or obstruction of the vena cava by thrombus, tumor, or membranes (73,74,75,76). Visualization of a caudate vein is over 90% specific for Budd-Chiari syndrome, although only 50% sensitive (77). The addition of Doppler to conventional ultrasonography increases sensitivity. Doppler is effective for evaluating not only the perihepatic vascular anatomy but also the direction of blood flow and site of obstruction. Loss of the normal triphasic wave variation in the vena cava or hepatic veins has a sensitivity of 88% for occlusion (78).

Computed tomography (CT) scan and magnetic resonance imaging (MRI) are complementary investigations to Doppler ultrasonography (79,80,81,82). Abnormalities seen may include nonvisualization of vessels or obstruction by thrombus (73,76,83,84,85). Acute thrombus may be demonstrated as an expanded nonenhancing vein (Fig. 40.1), whereas more chronic changes are suggested when vessels are narrowed or not visualized.

A "mosaic" pattern of abnormal parenchymal enhancement (Fig. 40.2), representing the effects of relative venous outflow obstruction, is commonly seen

in contrast-enhanced CT and MRI. This pattern is not specific for Budd-Chiari syndrome and can also be seen in severe right-sided heart failure. In cases of chronic venous outflow obstruction, parenchymal changes of nodular regeneration (including macroregenerative nodules) and cirrhosis may ensue (Fig. 40.3) (76,78,84,86). When portal hypertension develops, characteristic imaging findings (e.g., splenomegaly, ascites, collateral vessels) are often depicted as well.



▪ **Figure 40.1** Gadolinium-enhanced magnetic resonance imaging of a 67-year-old woman with Budd-Chiari syndrome and acute hepatic decompensation. Delayed post gadolinium images demonstrate expanded nonenhancing left hepatic vein (*arrow*), representing acute thrombus. A narrowed but enhancing middle hepatic vein is also shown (*arrowhead*). Vessel narrowing suggests prior thrombosis with recanlization. Portal vein thrombus and ascites were also present. Patient decompensated and underwent transplantation. Explant pathology confirmed acute left hepatic vein thrombus. The middle and right hepatic veins with chronic thrombotic changes and recanalization were also demonstrated.

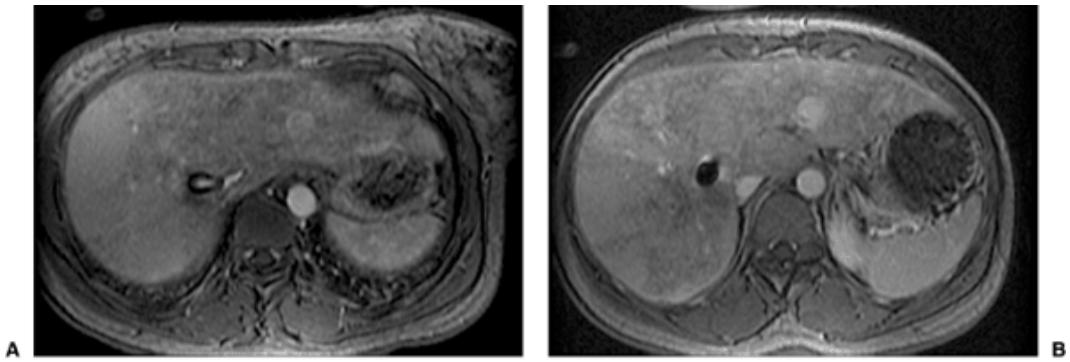
Venography remains the standard of reference for diagnosis and is key to planning optimal therapy. Typical venographic findings include narrowed, irregular hepatic veins with or without occlusive thrombi. Thrombi may be found either at the junction of the hepatic veins with the cava or just distal to the venous orifices (25,29,72). Replacement of hepatic veins with the classic "spider web" appearance, intrahepatic collaterals, and recanalized veins can also be appreciated (25,29,72,78,87). Angiographic assessment of the vena cava provides important information about the location and extent of obstruction, as well as its amenability to angioplasty and stenting (25,29,71,72,87). Both catheter-based and magnetic resonance venography (Fig. 40.4) can be used to depict stenoses or webs of the inferior vena cava. Local installation of thrombolytics may be performed at the time of venography in patients with fresh clots (less than 3 to 4 weeks) (88). Liver biopsy may also be obtained at the time of catheter venography. Furthermore, pressure measurements obtained at the time of venography can provide useful information before surgical decompression (25,29,71,72,87).

Liver biopsy

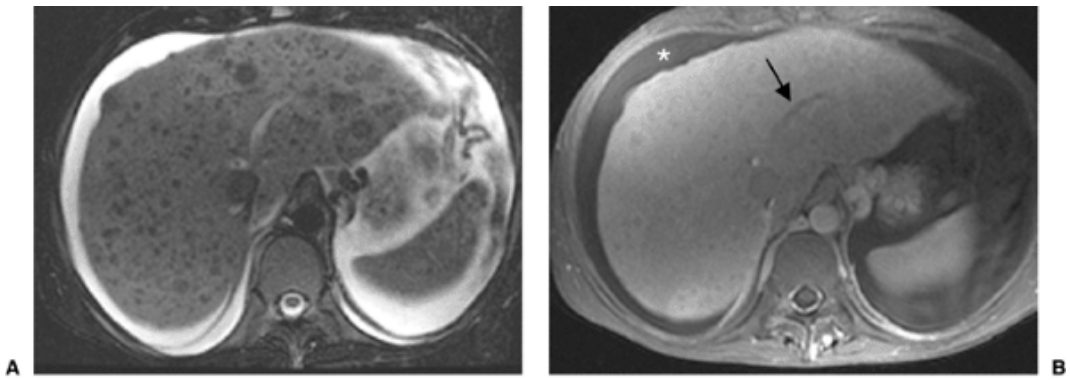
Although not mandatory, liver biopsy is complementary to medical imaging and clinical patient assessment. Generally, the diagnosis can be established by medical imaging. In select patients, biopsy may be required to distinguish Budd-Chiari syndrome from veno-occlusive disease or cirrhosis from other causes.

Biopsy specimens may establish the presence of fibrosis and cirrhosis and may also grade the severity of hepatocellular necrosis. However, caution should be used when interpreting results because sampling error is common. Bilobar biopsy may provide a higher diagnostic yield (87). However, it is not clear whether results from liver biopsy determine the prognosis (89,90,91). In one multivariate analysis, age, Child-Pugh score, responsiveness to diuretics, and creatinine were predictive of survival. Data from carefully documented

biopsies did not predict survival (90). A follow-up study found that diagnosis of acute or chronic Budd-Chiari syndrome, as opposed to acute on chronic injury only, was an important prognostic indicator for survival (91).



▪ **Figure 40.2** Gadolinium-enhanced magnetic resonance imaging (MRI) of a 57-year-old woman with chronic Budd-Chiari syndrome and prior transjugular intrahepatic portosystemic shunt. The MRI demonstrates heterogeneous (mosaic) enhancement in the left lobe **(A)** and portions of the right lobe **(B)**.



▪ **Figure 40.3** Magnetic resonance imaging of the liver of a 31-year-old man with long-standing Budd-Chiari syndrome. In the T2-weighted image **(A)**, liver parenchyma is abnormally edematous, with signal intensity elevation

(comparable to that of spleen). Multiple regenerative nodules characteristic of long-standing Budd-Chiari syndrome are hypointense to the edematous liver parenchyma. In the postgadolinium image **(B)**, nodular enhancement heterogeneity is shown. A portion of the thrombosed left hepatic vein is demonstrated (*arrow*). Ascites is also noted (*asterisk*).

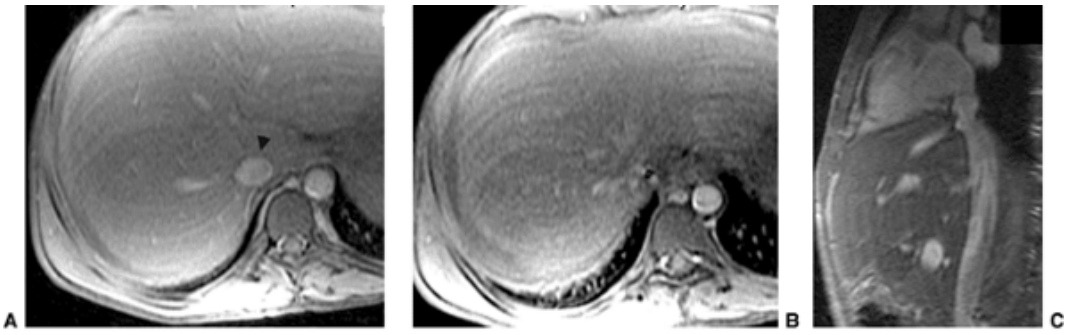
Management

In the absence of prospective natural history studies of untreated and unselected patients and randomized, controlled trials of treatment options, firm management recommendations cannot be made; however, some general principles have emerged. Anticoagulation is recommended for most patients. Thrombolytics may be of benefit in fresh thrombosis. Angioplasty with or without stenting may be considered for focal obstruction. If symptoms are progressive despite medical therapy, then decompressive therapy with TIPS or surgical shunting is advised. Some investigators recommend decompressive procedures early in the course or if extensive necrosis is seen on liver biopsy. Generally, liver transplantation is reserved for acute liver failure or decompensated cirrhosis.

Medical therapy

Early series demonstrated poor survival in untreated Budd-Chiari syndrome; most patients died within

3 years of diagnosis (25,72). More recent studies show improved survival. In one study, 5-year survival was 50% before 1985 and 75% afterwards (90). Widespread adoption of anticoagulation with warfarin is thought to be the cause of this improved trend. It seems prudent to recommend anticoagulation even in the absence of an identifiable prothrombotic state. It is possible that in some patients with myeloproliferative disorders, hydroxyurea and aspirin may be more appropriate (92). Patients with ascites should be placed on low-sodium diets and diuretics. Responsiveness to such measures is an independent predictor of survival (90,91). If ascites is not easily controlled after anticoagulation and medical therapy, patients should then undergo decompressive therapy.



▪ **Figure 40.4** Cardiac-gated bright-blood (cine) vascular imaging 2 cm below **(A)** and at **(B)** the level of the hepatic vein confluence of a 20-year-old man with lower extremity swelling and liver dysfunction. In **(A)**, a patent inferior vena cava (IVC) with flow is demonstrated (*arrowhead*). In **(B)**, the IVC is narrowed. Dark areas within IVC lumen represent turbulent (nonlaminar) flow. Cavogram (*not shown*) demonstrated IVC stenosis with thrombus, severely narrowing the lumen with a large pressure gradient. Angioplasty relieved both lower extremity symptoms and hepatic abnormalities. Repeat cine magnetic resonance imaging in the sagittal plane after angioplasty **(C)** demonstrates a patent IVC with mild structuring at the site of prior stenosis.

In carefully selected patients with clots no older than 3 or 4 weeks, the prompt administration of thrombolytics (e.g., streptokinase, urokinase, or recombinant tissue plasminogen activator) has been effectively used to dissolve thrombi and relieve hepatic congestion (88,93,94). Thrombolysis may be more effective when thrombolytics are administered locally into the hepatic vein and combined with angioplasty with or without stenting (88).

Interventional radiology

Percutaneous transluminal balloon angioplasty has been used to treat focal stenoses of the inferior vena cava and/or hepatic vein (95). Although excellent short-term results are achievable, sustained patency rates of only 50% are seen at 2 years (96). Wire-, laser-, or needle-assisted angioplasty is appropriate for stenoses refractory to standard techniques (97,98). For salvage of failed angioplasty or as primary therapy for focal stenoses, percutaneous intraluminal stenting is an option (19,96,97,98). More than 80% of stents remain patent 3 years after placement, with good control of symptoms (19,99). Stenting has also been used to treat intrahepatic inferior vena cava obstruction caused by compression from caudate lobe hypertrophy, which would otherwise preclude portacaval shunting. After stenting, portacaval shunting has been successfully performed (100).

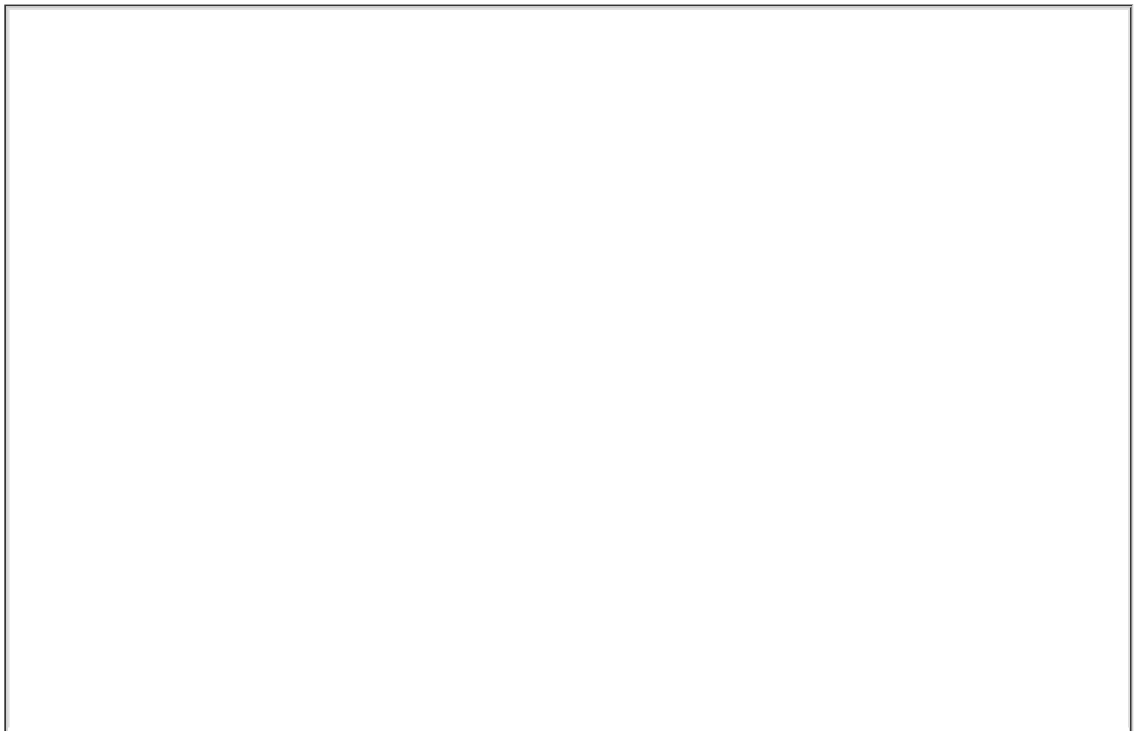
TIPS is being increasingly used as an alternative to decompressive surgical procedures because it is less invasive and does not carry the high perioperative mortality associated with surgical shunting. TIPS may have a role as a bridge to transplantation for patients with end-stage liver disease and ascites refractory to diuretics and sodium restriction (101,102,103,104) or in patients who present with fulminant hepatic failure (101,102,103,104,105,106). Recent series have reported on the use of TIPS after progressive, symptomatic liver disease with ascites despite anticoagulation and medical therapy. Control of symptoms, improvement of Child-Pugh class, and good 5-year survival without liver transplantation (74%) have been reported after TIPS (66,70). Survival after TIPS compares favorably with historical controls from surgical series. Shunt dysfunction often develops over time because of thrombotic occlusion. Therefore, surveillance with ultrasonography or angiography and chronic anticoagulation are recommended (70,102,106). Recent introduction of polytetrafluoroethylene-coated stents may dramatically reduce TIPS thrombosis. In one study, only 33% of patients with covered stents experienced dysfunction after TIPS within 1 year of the procedure, compared to 87% of patients with bare stents (107).

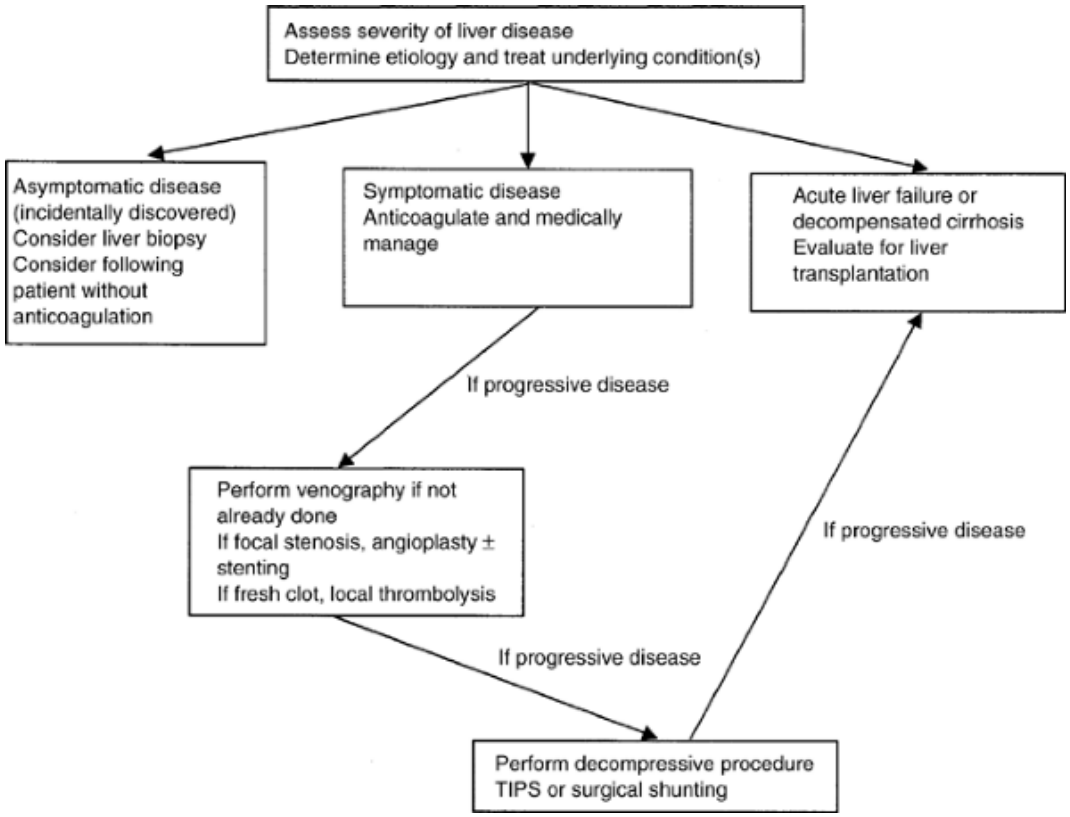
Surgical decompression

There is currently a divergence of opinion about whether surgical decompressive procedures should be offered early in the course of Budd-Chiari syndrome or should be reserved for patients who have progressive symptomatic disease despite medical therapy. One approach emphasizes the role of liver biopsy in determining which patients should undergo early surgical shunting. Anticoagulation alone is recommended if biopsy reveals only centrilobular congestion and sinusoidal dilatation, whereas surgery is reserved for those with hepatocyte necrosis (71,108). A second strategy advocates early surgical decompression, regardless of biopsy findings. Presumably, early relief of hepatic congestion may prevent ongoing necrosis and fibrosis. Such a strategy is supported by recent studies that found carefully collected data from liver biopsy to be not predictive of prognosis (89,90). Furthermore, in one retrospective study after adjustment for case severity, surgical shunting was associated with improved outcomes (89). If biopsy does not predict outcome, but performance of a surgical shunt does, then perhaps early surgery is preferable. However, caution must be exercised because no rigorous prospective study has been performed to test this strategy.

A third treatment approach has been increasingly used. Patients are first treated medically with anticoagulation and diuretics if ascites is present. If symptoms and serum tests of hepatic synthetic function do not improve within days for subacute Budd-Chiari syndrome and within weeks for slower presentations, then decompressive shunting is advised. Patients selected for surgery exhibited a nonsignificant trend toward improved survival (6). Similar treatment strategies using TIPS in place of surgical decompression have achieved excellent results (66,70). Success with TIPS has led to recommendations that decompressive surgery be relegated to third-line therapy. Moreover, surgical decompression is associated with high perioperative mortality (5% to 30%) and cannot be safely

performed for patients with Child-Pugh class B or C cirrhosis (19,50,70). The authors offer their own therapeutic algorithm (Fig. 40.5).





▪ **Figure 40.5** Proposed algorithm for management of Budd-Chiari syndrome. TIPS, transjugular intrahepatic portosystemic shunting.

A variety of portosystemic shunts is available to relieve sinusoidal hypertension of Budd-Chiari syndrome. Side-to-side portacaval shunts have been successfully used to convert the portal vein into an outflow tract (15,25,29,71,82,109). A pressure gradient of more than 10 mm Hg between the portal vein and the inferior vena cava is required for adequate shunt flow. For patients with compression of the intrahepatic portion of the inferior vena cava by a hypertrophic caudate lobe, inferior vena cava shunting may not be possible. Mesocaval shunts between the superior mesenteric vein and vena cava provide effective portal decompression for these patients (25,29,37,108). As with the side-to-side approach, caval pressure must be considerably lower than portal pressure. Shunt thrombosis remains a problem for 20% to 55% of patients who receive mesocaval shunts (15,37). Other shunts have been described, including splenocaval, cavoatrial, hepaticoatrial, and mesojugular shunts (110,111). Interposition synthetic grafts are also occasionally used. Finally, transatrial membranotomy with finger fracture or excision may be effective for patients with fenestrated membranes (29,37,112,113).

Liver transplantation

Liver transplantation is the preferred treatment for patients with Budd-Chiari syndrome and either acute liver failure or decompensated cirrhosis (15,29,37,87,109,114,115). Patients with significant liver disease who decompensate after receiving decompressive shunts or those with shunt failure

may be rescued with transplantation (112). Excellent 5-year patient and graft survival have been achieved, with rates similar to those for patients undergoing transplantation for other diseases (116,117). Transplantation is curative for protein C, protein S, and antithrombin III deficiency. All patients should receive indefinite anticoagulation for hypercoagulable states not curable by liver replacement (115). Aspirin and hydroxyurea effectively reduce platelet aggregation and number, respectively, and may be appropriate for preventing recurrent thrombosis after transplantation in patients with underlying myeloproliferative disorders (92,118). Patient selection is critical because individuals with short life expectancies due to underlying medical conditions are not appropriate for transplantation. However, most underlying myeloproliferative and other disorders associated with Budd-Chiari syndrome have near normal 10-year

life expectancies, which should not preclude transplantation (117). It is possible that immunosuppression could accelerate malignant transformation after transplantation, but this has not yet been reported.

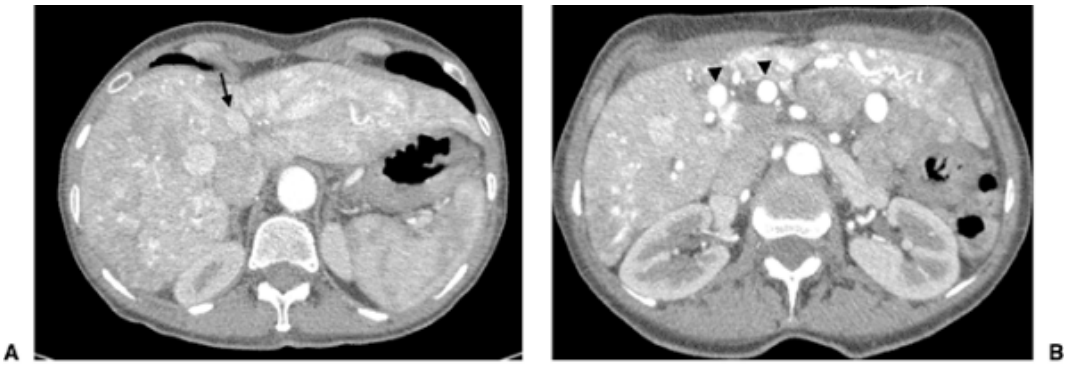
Liver Involvement in Hereditary Hemorrhagic Telangiectasia

HHT, also known as *Rendu-Osler-Weber syndrome*, is a multisystemic vascular disorder that variably affects the liver. Curacao criteria require presence of three of the four following criteria for definitive diagnosis: Recurrent and spontaneous epistaxis, multiple mucocutaneous telangiectasias, visceral arteriovenous malformations, and diagnosis of HHT in a first-degree relative (119). The disease is autosomal dominant and exhibits age-dependent penetrance. Identification of endoglin (*ENG*) and activin receptor-like kinase (*ALK-1*) gene mutations have allowed subclassification of HHT into types 1 and 2 (120,121). Both ENG and ALK-1 encode membrane glycoproteins expressed on vascular endothelial cells involved in vascular remodeling (122). Patients affected with HHT1 tend to have ENG mutations and are more likely to have pulmonary arteriovenous malformations. HHT2 is associated with ALK-1 mutations and is often clinically milder and more commonly associated with hepatic involvement.

Retrospective studies suggested radiologic evidence for hepatic involvement in only 8% to 30% of patients with HHT (123,124). However, a recent prospective study using multiphasic helical CT scan demonstrated hepatic vascular abnormalities, including arterioportal shunts, arteriosystemic shunts, telangiectasias, vascular masses, and parenchymal perfusion disorders, in up to 74% of 70 serial patients with HHT (125). Hepatic vascular abnormalities may be visualized with Doppler ultrasonography, CT angiography (Fig. 40.6), direct catheter angiography, or magnetic resonance angiography (126). Using contrast-enhanced CT or catheter angiography as the gold standard, sensitive and specific sonographic criteria for HHT have been defined. These include a dilated common hepatic artery (>7 mm) and the presence of intrahepatic hypervascularization on Doppler evaluation (127). Therefore, absence of these findings on a sonographic study may obviate the need for additional imaging. Furthermore, screening for hepatic involvement in HHT is not generally required because most hepatic lesions remain asymptomatic and do not require treatment (125,128).

There are several possible symptomatic presentations of HHT with hepatic involvement (Table 40.4). High-output cardiac failure presenting with dyspnea and edema may be related to extensive hepatic artery to hepatic vein shunting

(129). Portal hypertension resulting from shunting between the hepatic artery and portal vein may present with ascites or variceal hemorrhage (129). Hepatic encephalopathy may develop in the presence of portal vein to hepatic vein shunting (130). Finally, cholestasis and biliary abnormalities may result from ischemic injury related to hepatic artery to hepatic vein shunting (129). Symptomatic disease can often be managed conservatively. Hepatic arterial embolization of vascular lesions was initially used with some success, but more recent reports have highlighted the high mortality rates associated with postembolization hepatic and biliary necrosis (131). Hepatic arterial embolization can no longer be recommended for the treatment of hepatic HHT. Liver transplantation has been successfully performed despite technical difficulties with vascular reorganization (132). However, a recent report suggests the possibility that in some patients vascular abnormalities may reoccur after transplantation (133).



▪ **Figure 40.6** Contrast-enhanced computed tomography scan in a female patient with Rendu-Osler-Weber syndrome and hepatic involvement. In upper liver **(A)**, heterogeneous hypervascularity of the liver parenchyma is shown. Hepatic veins are markedly dilated (*black arrow*). At the level of porta hepatis **(B)**, the dilated tortuous proper hepatic artery is shown (*black arrowheads*).

Table 40.4. Hepatic Involvement of Hereditary Hemorrhagic Telangiectasia: Clinical Presentations

Clinical presentation	Type of shunting
High-output cardiac failure Dyspnea Edema	Hepatic artery to hepatic vein
Portal hypertension Ascites	Hepatic artery to portal vein

Variceal bleeding	
Hepatic encephalopathy	Portal vein to hepatic vein
Biliary abnormalities Cholestasis Cholangitis	Hepatic artery to hepatic vein

Peliosis Hepatis

Peliosis hepatis is characterized by blood-filled cystic cavities in the hepatic parenchyma lined by hepatocytes or endothelial cells (134). Similar lesions may also develop in the spleen. Peliosis has been well described in immunosuppressed patients, including those with HIV infection, tuberculosis, and cancer, and after solid organ transplantation (135,136,137). Medications associated with peliosis hepatis include anabolic and androgenic steroids, azathioprine, and cyclosporine (136,138).

Bartonella henselae, associated with cat-scratch disease, is the causative agent of bacillary peliosis hepatis in patients with HIV infection (135). Exposure to cat bites, scratches, and fleas are risk factors for acquisition of *Bartonella henselae* (135). This fastidious gram-negative bacillus has a granular and purple appearance on Warthin-Starry stain of affected hepatic specimens (134). Infection with the bacteria may also be demonstrated by blood culture, serologies, and polymerase chain reaction–based tests, although no single test is reliable. Some patients with bacillary peliosis hepatis also develop bacillary angiomatosis, characterized by vascular lesions affecting the skin (most commonly red or purple papules), lymph nodes, bones, and central nervous system.

Imaging reports of peliosis hepatis are largely limited to isolated case reports (139,140,141,142). On sonography, vague areas of slightly decreased heterogeneous echotexture have been described. On unenhanced CT scan, irregular areas of low attenuation are shown. On MRI, lesions are usually of low-signal intensity on T1-weighted imaging and high-signal intensity on T2-weighted imaging. However, appearance on MRI may vary if lesions are complicated by hemorrhage, with variable T1 brightening. On both CT and MRI, dynamic contrast enhancement demonstrates an early central enhancement, with gradual centripetal fill-in. This pattern is distinct from that of other vascular liver lesions, including hemangioma, adenoma, and focal nodular hyperplasia and may be the key to diagnosis.

Peliosis hepatis is often an incidental finding. Among patients with HIV infection, fever, lymphadenopathy, anemia, elevated alkaline phosphatase level, and lower CD4 counts are more common in affected patients than in controls (143). Treatment of bacillary peliosis hepatis consists of several months of

administration of erythromycin (500 mg four times daily) or an alternative macrolide. Nonbacillary peliosis is best treated by stopping the offending medication or addressing the underlying etiology. Performance of liver transplantation for treatment of severe peliosis hepatis has been reported (144).

Summary

Budd-Chiari syndrome is an uncommon disease associated with thrombotic obstruction of the terminal hepatic venules, hepatic veins, vena cava, and/or right atrium. Sinusoidal congestion and centrilobular necrosis occur as a consequence of venous outflow obstruction and can lead to fibrosis and cirrhosis. Most patients present with chronic signs and symptoms of portal hypertension, although some may present subacutely or even acutely. Doppler sonography is an excellent screening test for evaluating the patency of the major hepatic veins and vena cava. CT scan and MRI are complementary studies. Venography remains the standard of reference for the diagnosis of venous occlusion and may either show the classic "spider web" appearance of hepatic vein obstruction or demonstrate intrahepatic or subcapsular collaterals. Liver biopsy and pressure measurements made during venography may also guide treatment selection.

Rigorous studies are not available to derive definitive treatment algorithms. Generally, anticoagulation is offered to all patients. Diuretics and low-sodium diets are prescribed for those with ascites. If symptoms and hepatic synthetic function do not resolve after medical therapy, then decompressive procedures are offered. Some investigators recommend performance of surgical shunting or TIPS early in the course of disease. Thrombolytic therapy may be useful for fresh thrombosis. Angioplasty with or without stenting may treat focal stenoses. TIPS can effectively decompress hepatic sinusoids with symptomatic improvement. Surgical shunting has been used successfully for those with adequate hepatic reserve, although perioperative mortality can be high. Liver transplantation is preferred for patients with acute liver failure or decompensated cirrhosis. Survival after transplantation is similar to that of patients undergoing transplantation for nonmalignant cirrhosis of other causes.

HHT is characterized by epistaxis, mucocutaneous telangiectasias, and visceral arteriovenous malformations. Whereas liver transplant has been demonstrated frequently, patients with hepatic lesions are generally asymptomatic. Possible symptoms can include high-output cardiac failure, complications of portal hypertension, hepatic encephalopathy, and symptoms related to cholestasis. Management is most often conservative, and hepatic arterial embolization has been associated with unacceptably high mortality.

Peliosis hepatis is often an incidental finding. Characteristic blood-filled cystic cavities may occur in the setting of immunosuppression or HIV infection. *Bartonella henselae* is the cause of bacillary peliosis hepatis in patients with HIV infection. Generally, treatment consists of addressing the underlying cause of immunosuppression or treating *Bartonella* infection.

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Chapter 41

The Liver in Circulatory Failure

Mitchell L. Shiffman

Key Concepts

- A dual blood supply protects the liver from developing ischemic injury during periods of systemic hypotension. When ischemia does occur it is typically limited to zone 3 of the hepatic lobule and resolves rapidly and spontaneously when systemic blood pressure is restored.
- Ischemic hepatitis occurs in the setting of acute and severe hypotension and is associated with an abrupt and profound elevation in the levels of serum liver transaminases, which return to the normal range within several days. The serum bilirubin level rises and peaks 3 to 5 days after the peak in serum liver transaminase levels.
- Occlusion of the hepatic artery or one of its branches will lead to infarction of the area supplied by the occluded vessel. Large hepatic infarcts may become infected and develop into a hepatic abscess.
- The hepatic artery provides the only blood supply to the biliary ductal system. Injury to or occlusion of hepatic arterial branches may cause ischemic cholangiopathy. This results in stricture formation of bile ducts, which is often associated with dilatation proximal to the stricture.
- Passive hepatic congestion may develop as a consequence of right ventricular failure and elevated central venous pressure. Ascites may develop from passive hepatic congestion in the absence of cirrhosis. Cardiac cirrhosis may develop after a prolonged period of passive hepatic congestion.
- Passive hepatic congestion alone is only rarely associated with hepatic synthetic dysfunction even when cardiac ascites or cardiac cirrhosis is present. Liver dysfunction in this setting is most commonly the result of biventricular failure in which periods of hypotension and hepatic ischemia are superimposed on chronic hepatic congestion.

The liver has a dual blood supply and receives blood from both the hepatic artery and portal vein. These blood supplies mix within hepatic sinusoids and subsequently drain through multiple hepatic veins (1). Blood flow through the liver is therefore somewhat protected from the acute and chronic changes in cardiac output and systemic blood pressure. However, severe acute and/or prolonged changes in cardiac function may lead to hepatic dysfunction (2). Furthermore, the type of cardiac dysfunction may affect the liver in different ways. For example, cardiovascular failure associated with hypotension could

affect hepatic arterial blood flow and oxygen delivery to the liver (3). If abrupt, severe, and self-limited, this could lead to a marked elevation in levels of liver aminotransferases (ATs) without affecting hepatic function and without long-term sequela. If less severe but chronic, only minor elevations in liver AT levels may occur but progressive fibrosis secondary to chronic hepatic ischemia may result. In contrast, cardiac dysfunction secondary to right ventricular failure may lead to an elevation in hepatic venous pressure, passive hepatic congestion, and

the development of ascites with or without cirrhosis (4). Hepatic injury caused by acute or chronic circulatory failure may therefore present with a wide spectrum of biochemical, histologic, and pathophysiologic patterns.

This chapter focuses on liver injury caused by acute or chronic cardiac disease secondary to left ventricular dysfunction, right ventricular failure, valvular heart disease, constrictive pericarditis, congenital heart disorders, or circulatory failure secondary to heatstroke. Disorders of the liver that result from cardiac dysfunction include passive hepatic congestion, ischemic hepatitis, ischemic cholangiopathy, and hepatic infarction. The liver may also be injured or liver chemistries may become abnormal by many other disorders that affect hepatic blood flow. These disorders, which include hepatic vein thrombosis (see Chapter 40), portal vein thrombosis (see Chapter 40), hepatic veno-occlusive disease (see Chapter 40), sepsis (see Chapter 50), and various forms of immunologic disorders associated with vasculitis (see Chapter 11), will not be discussed in this chapter.

Anatomy and Physiology of Hepatic Blood Flow

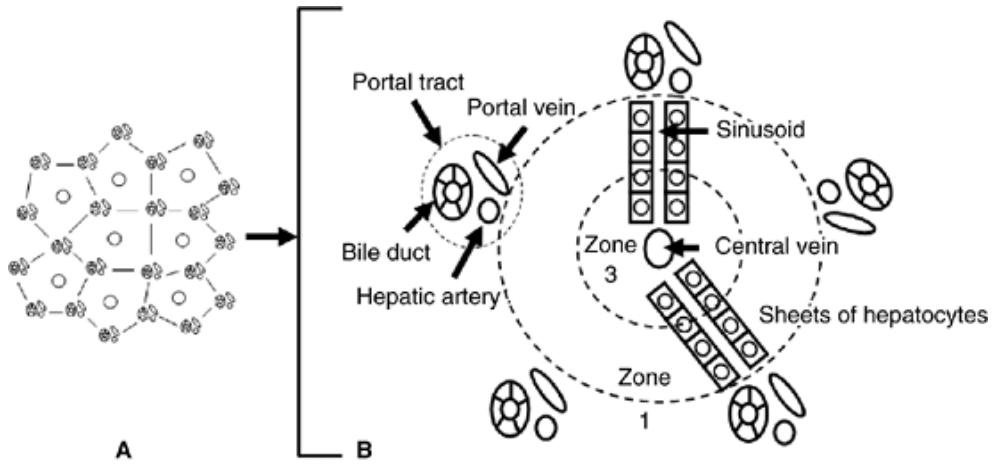
A detailed description of hepatic anatomy and the physiology of hepatic blood flow is provided in Chapter 7. This is only briefly reviewed here, with special emphasis on the pathophysiologic factors associated with circulatory failure.

The liver has a rich blood supply derived from two sources (1). Two thirds of the total hepatic blood flow enters the liver through the portal vein. Portal blood contains large amounts of nutrients absorbed from the intestine, particularly after a meal, including glucose, amino acids, water-soluble vitamins, and triglycerides, but a relatively low oxygen tension. The remaining one third of hepatic blood flow is supplied by the hepatic artery. Hepatic arterial blood contains little nutrients but is rich in oxygen. More than half of the oxygen delivered to the liver originates from the hepatic artery, which is also the only blood supply for both the extrahepatic and intrahepatic biliary ductal systems. As a result, the liver and, particularly, the biliary ductal system are more susceptible to ischemic damage after an abrupt reduction in hepatic arterial blood flow than portal blood flow. In animal studies, ligation of the hepatic artery was associated with massive hepatic necrosis and death, whereas ligation of the portal vein was not (5). Inadvertent ligation of hepatic arterial branches during laparoscopic cholecystectomy (6) or purposeful embolization of hepatic arterial branches for treatment of hepatocellular carcinoma (HCC) (7) may lead to hepatic infarction of the segment supplied by the occluded vessel. Hypotension may also result in subsegmental, segmental, or global hepatic infarction (8). Patients with portal vein thrombosis, in which the only hepatic blood supply is through the hepatic artery, are particularly susceptible to hepatic failure secondary to a disruption in hepatic arterial blood flow. This may occur after chemoembolization of HCC or simply with hypotension.

The functional unit of the liver is the hepatic acinus or lobule. This is a roughly

pentagonal-shaped structure with a single central hepatic vein bound by five portal tracts (Fig. 41.1). Each portal triad contains a branch of the hepatic artery, portal vein, and a bile duct. Sheets of hepatocytes, one to two layers in thickness and lined

by hepatic sinusoids, stretch between the portal triads and central vein. Both portal and hepatic arterial blood enter the liver lobule through portal tracts, zone 1 of the hepatic acinus in the Rappaport classification (9). Blood from both of these sources then enters and mixes within hepatic sinusoids, flows to the center of the hepatic lobule (Rappaport zone 3), and exits the liver through a branch of the hepatic vein. As a result, zone 1 of the hepatic acinus contains oxygen- and nutrient-rich blood, and as this flows through the sinusoids, it is gradually depleted of both nutrients and oxygen. Zone 3 contains the smallest amount of nutrients and oxygen and is therefore the most susceptible to ischemic injury.



▪ **Figure 41.1 A:** Schematic drawing of a section of liver. The functional subunit of the liver is the hepatic lobule or acinus, a roughly pentagonal-shaped structure with a central vein in the center and portal triads at each corner. Each portal triad is shared by several hepatic lobules. **B:** Schematic drawing of a hepatic lobule or acinus. Each portal triad contains a branch of the hepatic artery, portal vein, and bile duct. Sheets of hepatocytes extend between the central vein and portal triads at the edges of the lobule. Hepatic arterial and portal venous blood enters the acinus through the portal triad mix and then flow through hepatic sinusoids to the central vein. Zone 1 of the lobule lies at the periphery of the hepatic acinus (*outer dashed circle*). Zone 3 of the lobule lies in the center of the acinus (*inner dashed circle*).

Blood flow into the hepatic lobule is self-regulated by the hepatic microvasculature. Blood entering the liver through the low-pressure portal vein is slow, constant, only marginally affected by system blood pressure, and unregulated. In contrast, high-pressure hepatic arterial flow is tightly regulated so that total blood flow, the sum of portal and arterial blood flow, entering the liver lobule remains within a relatively narrow range (10). The buffering response of hepatic arterial blood flow to changes in portal blood flow is regulated by adenosine, a potent vasodilator of arterial smooth muscle cells (11,12). Adenosine is continuously secreted into the portal triad, and its local

concentration is controlled by the blood entering the hepatic lobule. Therefore, as portal blood flow increases, the local adenosine concentration is diluted, hepatic arterioles constrict, and arterial blood flow declines. Alternatively, as portal blood flow declines, the local concentration of adenosine increases, hepatic arterioles dilate, and arterial blood flow increases. The response of hepatic arterial smooth muscle to adenosine is maintained in patients with cirrhosis (13). Infusion of adenosine has been shown to reduce hepatic ischemic injury in experimental animals (14,15) and reduce ischemic injury following reperfusion after liver transplantation (16). Adenosine appears to act locally by the synthesis of nitric oxide (17,18).

Ischemic Hepatitis

Ischemic hepatitis refers to the disorders that cause liver injury by reducing oxygen delivery to the liver (19). This is most commonly seen in the setting of severe hypotension and global hepatic hypoperfusion (Table 41.1). In many patients ischemic hepatitis results from a severe acute decline in cardiac output associated with myocardial infarction, pulmonary embolus, or congestive heart failure. In other patients ischemic hepatitis may result from hypovolemia associated with massive hemorrhage, dehydration and heatstroke (20), sepsis, or hypoxia associated with acute respiratory failure. When associated with severe hypotension and shock, the term *shock liver* is frequently utilized.

Table 41.1. Causes of Ischemic Hepatitis

HYPOTENSION
Cardiac dysfunction
Right of left ventricular myocardial infarction
Cor pulmonale
Pulmonary embolus
Acute exacerbation of congestive heart failure
Hypovolemia
Hemorrhage with or without preceding trauma
Dehydration
Burns associated with severe dehydration
Miscellaneous causes
Sepsis
Heatstroke
Sickle cell crisis
HYPOXIA
Acute respiratory failure or acute exacerbation of chronic respiratory disease
Obstructive sleep apnea

Pathophysiology

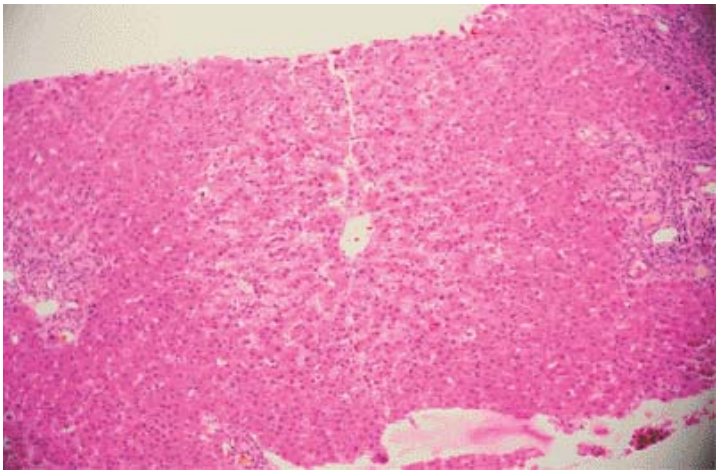
Ischemic hepatitis is not a true hepatitis because inflammation is uniformly absent on histologic examination. Rather, the hallmark of this disorder is zone 3 hepatic necrosis along with variable degrees of hepatic lobular collapse, depending on the duration and severity of the insult (Fig. 41.2). Fibrosis is characteristically absent unless there is a separate preexisting underlying chronic

liver disorder or a long-standing circulatory disease associated with passive congestion, hypovolemia, or hypoxia. In patients with right ventricular infarction, changes associated with both acute passive congestion (see subsequent text) and ischemic hepatitis may be present. The histologic features of ischemic hepatitis resolve spontaneously, hepatocytes regenerate, and liver histology returns to normal in most patients. In patients with preexisting fibrosis and/or other histologic features of a chronic liver disorder, these changes either remain or recur after resolution of ischemic hepatitis.

Clinical Features and Outcome

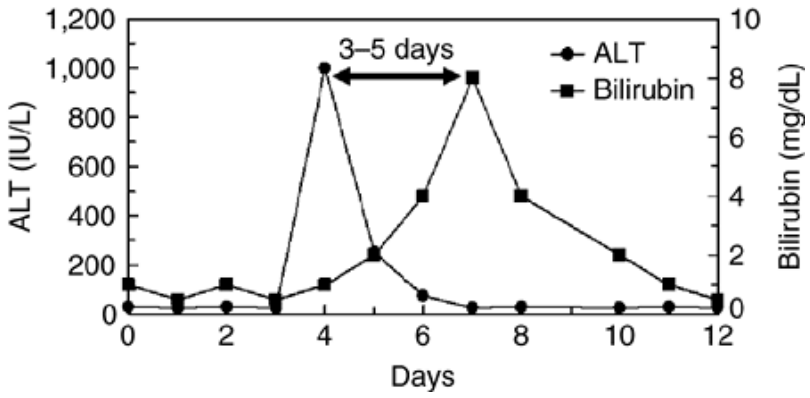
The clinical hallmark of ischemic hepatitis consists of an abrupt elevation in the levels of serum liver ATs, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and lactate dehydrogenase (LDH), which peak within 1 to 3 days of an episode of severe hypotension (Fig. 41.3). This is readily detected because patients with ischemic hepatitis typically are acutely ill and hospitalized, and undergo frequent biochemical testing of their renal, liver, and cardiac function. The peak in AT levels is typically in the 500 to 1,500 IU/L range, and these values return to normal within another 3 to 7 days as long as the hypotensive event is effectively treated and/or resolves (3,21,22,23).

The serum bilirubin level begins to rise 1 to 2 days after the elevation in serum AST and ALT levels and peaks 3 to 5 days after the maximum rise in liver AT levels. The maximal value for serum bilirubin observed in patients with ischemic hepatitis will vary greatly and depend on the inciting event and the presence of any underlying chronic liver disorder. Serum alkaline phosphate value may remain in the normal range or increase by only mild to moderate amounts. The international normalized ratio (INR) usually remains within normal limits. However, in particularly severe cases of ischemic hepatitis the patient may develop a mild prolongation in INR that rapidly corrects after administration of vitamin K. Changes in mental status are common in patients with ischemic hepatitis. However, this is usually secondary to cerebral hypoperfusion and hypoxia associated with the acute cardiocirculatory process. True hepatic encephalopathy associated with an elevation in serum ammonia level is distinctly rare.



▪ **Figure 41.2** Histology of ischemic hepatitis. There is necrosis and

ballooning degeneration of hepatocytes adjacent to the central vein, zone 3 of the hepatic lobule, with preservation of hepatocytes in zone 1 and portal structures.



▪ **Figure 41.3** Pattern of serum alanine aminotransferase (ALT) and bilirubin level variation in patients with ischemic hepatitis. There is an abrupt and marked rise in serum aspartate aminotransferase (AST—not shown) and ALT levels to values of 200 to 1,000 IU/L. This is followed by a delayed rise in serum bilirubin level, which usually reaches a maximum 3 to 5 days after the peak in serum AST and ALT levels.

The possibility that the abrupt rise in serum liver AT levels in a patient with presumed ischemic hepatitis may actually result from an acute viral or nonviral hepatitis should always be considered. However, this rapid rise and fall in AST and ALT levels is unusual in acute viral hepatitis in which the transaminase levels return to normal over weeks rather than days. In addition, acute viral or nonviral hepatitis is frequently symptomatic and is associated with nausea, vomiting, anorexia, malaise, and/or right upper quadrant discomfort (19,24). These symptoms are only rarely present in patients with ischemic hepatitis. Despite this, serologic studies to exclude viral hepatitis A, B, and C may need to be performed in some cases. Nonviral causes of acute hepatitis that could present with an abrupt marked elevation in the values serum liver ATs include Wilson disease and autoimmune hepatitis (25,26). In most cases these disorders can be excluded simply by evaluating the clinical presentation, although appropriate serologic testing may also rarely be required. Finally, ischemic hepatitis is frequently associated with signs or symptoms of hypoxic or hypotensive injury to another organ; the one most commonly affected is the kidney. Therefore, a parallel rise in serum creatinine level is frequently observed in patients with ischemic hepatitis (3,23).

Although the inciting event is readily apparent in most patients with ischemic hepatitis, a transient or subclinical hypotensive event may occasionally trigger this process and lead to uncertainty in the diagnosis. This is particularly common in patients with chronic right or left ventricular failure associated with chronic

hypotension and/or passive hepatic congestion in which the liver is quite sensitive to even minor changes in hepatic arterial blood flow (24,27,28). Ischemic hepatitis may also be difficult to recognize in patients with hypoxia secondary to exacerbations in chronic pulmonary disease or sleep apnea in the absence of hypotension (29).

The severity and prognosis of ischemic hepatitis is primarily dependent on the outcome of the inciting cardiocirculatory event. If the cause for hypotension is rapidly corrected the hepatic event is self-limited and resolves promptly. In contrast, if hypotension is extremely severe and/or persists despite prolonged resuscitative efforts multiorgan failure and death is likely to ensue. Ischemic hepatitis may lead to fulminant hepatic failure and death, but this is uncommon and typically occurs only in the setting of chronic congestive heart failure associated with passive hepatic congestion or cardiac cirrhosis (27) or in patients with cirrhosis from another form of chronic liver disease. The later situation is most frequently

encountered when a patient with cirrhosis develops hypotension secondary to massive variceal hemorrhage and/or sepsis. Ischemic hepatitis in patients with cirrhosis is associated with a mortality in excess of 60% (30,31).

Treatment

The treatment of ischemic hepatitis is directed at restoring normal circulatory hemodynamics and correcting the cause of the hypotensive event. No specific therapy for enhancing hepatic recovery from ischemic hepatitis currently exists. One study has suggested that intravenous low-dose dopamine may augment hepatic blood flow but the benefit of this on hepatic recovery from ischemic hepatitis has not been demonstrated (32). Adenosine has been utilized in animal models but not in humans except to reduce reperfusion injury in donor liver grafts (14,15,16).

Hepatic Infarction

Hepatic infarction represents the most severe form of ischemic hepatitis. Whereas ischemic hepatitis is associated with a global decline in hepatic blood flow and/or oxygen delivery secondary to a cardiocirculatory or other systemic process and is typically reversible, hepatic infarction occurs when blood flow to the liver, a particular lobe, segment, or subsegment, is interrupted secondary to occlusion of the hepatic artery or one of its branches. The involved segment of liver has no ability to recover and heals through the process of scar formation (33). Fortunately, only a branch or sub-branch of the hepatic artery is typically involved and the amount of liver rendered ischemic is limited to a segment or subsegment. In these cases, global hepatic function is only marginally impaired. However, if a major branch of the hepatic artery is occluded and the amount of liver rendered anoxic is too large the patient may develop acute liver failure and die without emergent liver transplantation.

The various etiologies of hepatic infarction are listed in Table 41.2. Hepatic infarction may occur when the artery is injured during liver transplantation (34,35), cholecystectomy, (6,36) placement of a transjugular intrahepatic portosystemic shunt (TIPS) (37), administration of intra-arterial chemotherapy or purposeful embolization of the artery for treatment of HCC or cancer metastasis to the liver (7,38). Hepatic infarction may also result from a hypercoagulable state associated with vasculitis or polyarteritis (39,40,41,42,43), septic emboli

(44), sickle cell crisis (45,46), polycythemia vera (33), and the use of oral contraceptives (47) and is seen in patients with toxemia of pregnancy (48,49). Severe arteriolar vasospasm associated with cocaine use may also lead to hepatic infarction (50,51).

Table 41.2. Causes of Hepatic Infarction

IATROGENIC ARTERIAL INJURY
Liver transplantation
Laparoscopic or open cholecystectomy
Intra-arterial chemoembolization or chemoinfusion
Placement of transjugular intrahepatic portosystemic shunt
SYSTEMIC DISORDERS
Polyarteritis
Systemic lupus erythematosus
Sickle cell crisis
Emboli from infectious endocarditis
HYPERCOAGULABLE STATES
Abnormalities in coagulation system
Polycythemia vera
MISCELLANEOUS CAUSES
Aortic dissection
Toxemia of pregnancy
Cocaine intoxication

Pathophysiology

Hepatic infarction represents the most extreme form of ischemic injury to the liver, in which complete occlusion of the arterial supply renders the involved segment anoxic. Histologically there is complete coagulative necrosis of the entire hepatic acinus including all hepatocytes and portal structures that lie within the center of the involved segment. This is surrounded by a zone of inflammatory reaction. The periphery of the involved segment contains a zone of partial necrosis that closely resembles that seen in ischemic hepatitis, necrosis within zone 3 of the hepatic lobule with preservation of zone 1 structures (36). This area likely receives partial blood flow from an adjacent segment and will recover both histologically and functionally.

Clinical Features and Outcome

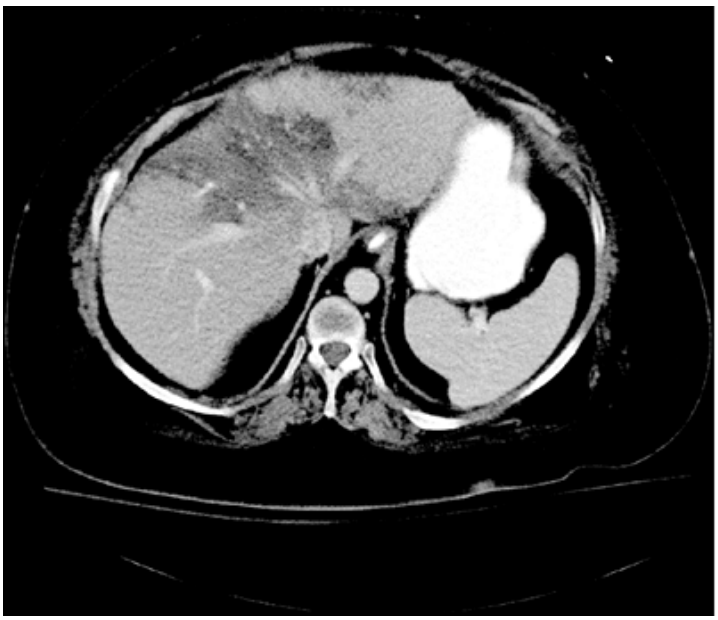
Patients who develop hepatic infarction may be asymptomatic if the occlusion occurs during a surgical or invasive procedure. In such cases the hepatic infarction may be suspected when liver AT levels are found to be markedly elevated after the procedure. In other cases, particularly if liver chemistries are not monitored and the infarction is of limited size, the diagnosis may be missed and the infarction will only be identified incidentally if and when an imaging study is performed. In nonsurgical settings patients may experience right upper quadrant pain of variable intensity that radiates to the back or right shoulder and is associated with fever, nausea, and vomiting (36).

Leukocytosis is common and there is a dramatic rise in serum liver AT levels

similar to what is observed in ischemic hepatitis. The serum bilirubin level may or may not rise depending on the size of the infarcted area. If a major branch of the hepatic artery is involved a large proportion of the liver will become infarcted and liver failure may occur (52).

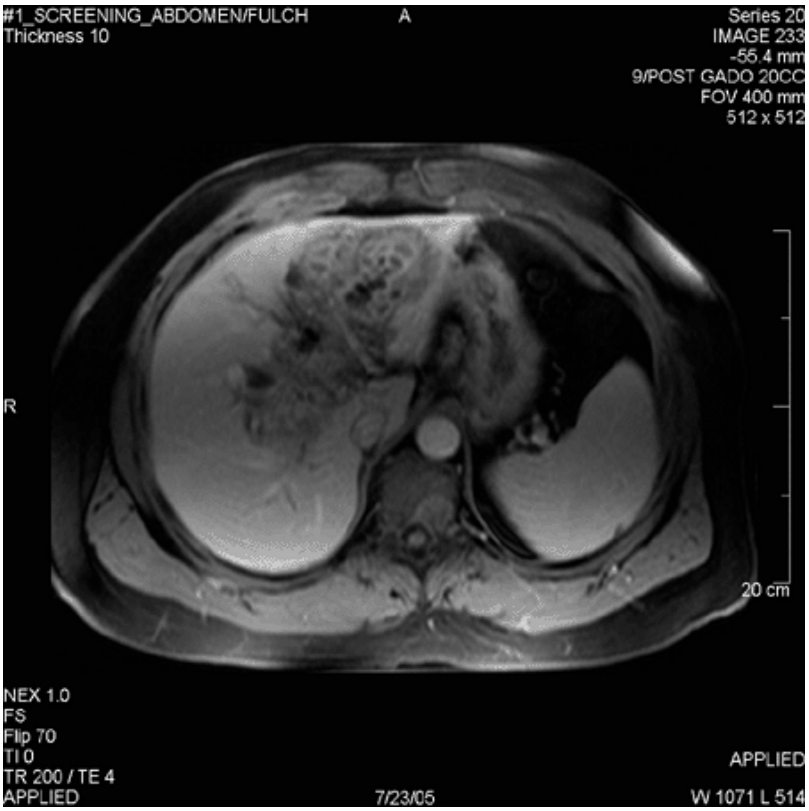
The diagnosis of hepatic infarction is confirmed with radiographic studies (53,54,55,56). If recognized acutely, ultrasound may demonstrate a focal hypoechoic area, and if the occlusion is of a large hepatic artery, Doppler flow ultrasonography may demonstrate the absence of flow in the involved vessel. Ultrasound is unlikely to detect the absence of flow in a smaller subsegmental branch of the hepatic artery. Abdominal computed tomography (CT) scan demonstrates a segmental area of low attenuation. The lesion may be wedge shaped and extend outward to the periphery of the liver (Fig. 41.4). Round or oval infarcted lesions are usually located within the center of the liver, or the lesion may be irregular and follow the course of the intrahepatic vasculature and biliary tracts (53,54). In such cases, marked biliary dilatation in the involved area occurs as the lesion heals (Fig. 41.5). With magnetic resonance (MR) imaging the lesion has diminished T1 and increased T2 signal intensity. MR angiography may also demonstrate the area of occlusion if a sufficiently large branch of the hepatic artery is involved (56,57).

It is rarely necessary to perform biopsy in an area of hepatic infarction. The diagnosis is based on the clinical history and radiographic findings. There is frequently a history of gallbladder, biliary tract, or hepatic surgery; a recent angiographic infusion to the involved area; a known hypercoagulable state; or another associated event (Table 41.2). In cases in which the diagnosis is uncertain and the lesion is radiographically atypical in its appearance, an ultrasound-directed biopsy may be useful. The major short-term concern is that the infarcted area will become infected and develop into an hepatic abscess. This is most commonly encountered after embolization of a hepatic tumor (38,58,59). In cases in which abscess formation is suspected broad-spectrum antibiotics should be initiated and needle aspiration of the lesion performed.



▪ **Figure 41.4** Computed tomography scan of a patient with a segmental

hepatic infarct. Note the wedge-shaped region of infarction that extends from the center of the liver to the periphery and the liver capsule.



▪ **Figure 41.5** Magnetic resonance image of a patient who developed an hepatic infarction and ischemic cholangiopathy after an episode of severe acute cholecystitis and cholecystectomy. Note that the area of infarction follows the course of the biliary tree and is associated with dilatation of bile ducts. Hepatic arteriogram (not depicted) demonstrated occlusion of intrahepatic branches of the left and middle hepatic artery corresponding to the area of infarction and cholangiopathy.

Treatment

No specific treatment for hepatic infarction is required. However, if the infarcted area is large the likelihood for abscess formation is increased and such patients should be closely monitored for signs of sepsis and/or treated with empiric antibiotics (58,59). Abscess formation is extremely common in patients who develop hepatic infarction after liver transplantation and is the major reason why such individuals undergo emergent retransplantation (34,35). Patients with massive hepatic infarction and liver failure should be considered candidates for emergent liver transplantation. Finally, if the cause for the infarction is not readily apparent a diagnostic evaluation for a source of emboli or an underlying hypercoagulable state is warranted.

Ischemic Cholangiopathy

The bile ducts receive their blood supply exclusively from branches of the hepatic artery and are therefore more sensitive to alterations in hepatic arterial flow than is the liver parenchyma, which also has a portal blood supply (1). Hepatic arterial injury or occlusion associated with hepatic infarction or profound hypotension associated with ischemic hepatitis frequently causes injury to the biliary ductal system. However, ischemic cholangiopathy may also occur in the absence of hepatic ischemia. Ischemic bile duct injury is most commonly observed after cholecystectomy, biliary tract surgery, hepatectomy, and liver transplantation but may also be caused by any of the etiologies listed in Table 41.2 (60,61,62,63,64).

Ischemic injury to the bile ducts results in the development of biliary strictures. This is commonly accompanied by dilatation of the biliary tree proximal to the lesion. Typically there is only a single focal stricture. However, in some cases the stricture may be quite long or there may be multiple strictures with intervening dilatation. In some cases these strictures may resemble those seen in cholangiocarcinoma or primary sclerosing cholangitis (PSC). However, most ischemic bile duct strictures have a smooth symmetric appearance and are confined to a focal segment of the liver. This is unlike that seen in patients with PSC, in which the strictures tend to be located throughout the liver. The development of an ischemic biliary stricture may be asymptomatic and only recognized when imaging studies are performed to evaluate the liver for an elevation in alkaline phosphatase level. The serum bilirubin level is usually normal. However, a mild to moderate elevation in serum bilirubin level or jaundice may develop if the stricture totally occludes the bile duct or is located in a major large bile duct.

Patients in whom ischemic cholangiopathy is suspected should undergo extensive imaging of the biliary ductal system with either an MR or endoscopic cholangiogram and serologic testing to exclude PSC (see Chapter 23) and/or cholangiocarcinoma (see Chapter 4). Placement of an endoscopic biliary stent or surgical revision of the bile duct is indicated if the bilirubin level is elevated. In an asymptomatic patient, liver biopsy of the involved lobe proximal to the stricture is frequently useful because this can identify histologic changes consistent with bile duct obstruction and patients at risk for developing secondary biliary cirrhosis. Intrahepatic bile duct dilatation and duplication proximal to the stricture has been identified histologically (65). Placement of an endoscopic stent should probably be performed in such patients, although no data currently exists to demonstrate that this reduces the risk for developing biliary cirrhosis. One study in a group of pediatric liver transplant recipients has suggested that the use of ursodeoxycholic acid in addition to stenting may prevent secondary biliary cirrhosis (62). In the liver transplant recipient, hepatic artery thrombosis in the immediate post-transplantation period is associated with extensive stricture formation of the biliary tree, intrahepatic abscess formation, and graft failure and is therefore an indication for emergent retransplantation (34,63). The development of biliary strictures months to years after liver transplantation is best treated endoscopically as in the nontransplantation setting.

Heatstroke and the Liver

Heatstroke is a severe systemic and potentially fatal disorder with a mortality

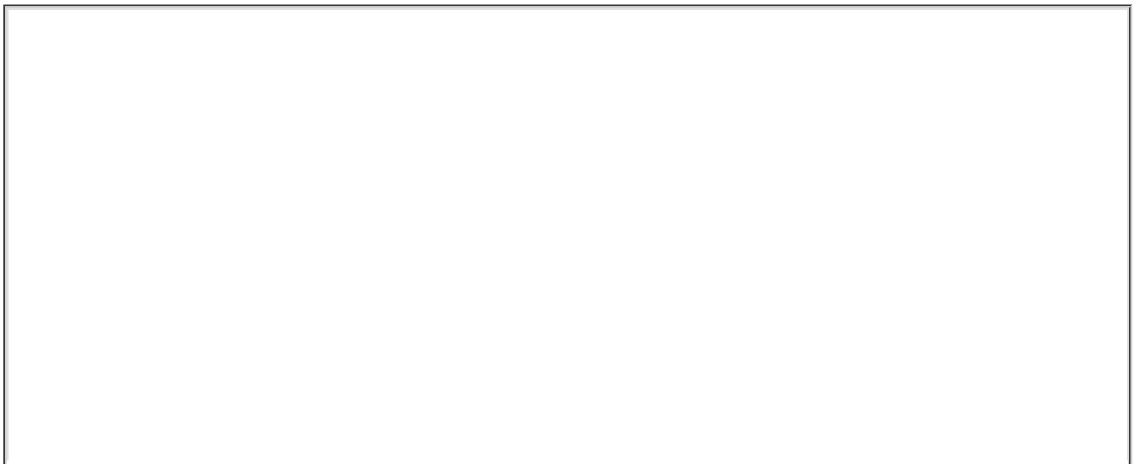
rate approaching 25% (20,66,67). The syndrome is characterized by hyperexia (a body temperature above 41°C), profound hypotension, neurologic impairment, heat-induced tissue injury, sepsis, disseminated intravascular coagulation, and multiorgan failure. The liver is frequently injured by heatstroke; excessive body temperature itself causes hepatic necrosis, and severe hypotension is associated with a superimposed ischemic hepatitis. Serum AT and LDH levels are markedly elevated in patients with heatstroke. However, the elevation in AST level is frequently much greater than the elevation in ALT level because of thermal injury to muscles, the brain, and kidneys (20,66,67). The elevation in serum ALT level is rarely greater than 20 times the upper limit of normal, and bilirubin level frequently remains normal or is only mildly elevated. The peak in serum AT levels, like that observed in ischemic hepatitis, occurs 1 to 2 days after the event. However, as opposed to ischemic hepatitis, serum levels of liver ATs recover much more slowly—over weeks rather than days (20). Elevations in serum ALT levels to values greater than this and/or a progressive rise in serum bilirubin level is associated with high mortality.

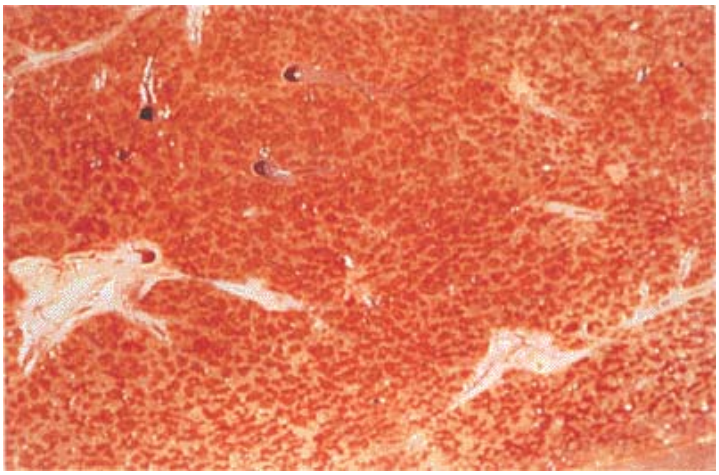
The histologic changes observed in patients with heatstroke consist of zone 3 necrosis, similar to that observed in patients with ischemic hepatitis. However, steatosis, dilatation of hepatic and portal veins, sinusoidal dilatation, and cholangiolar proliferation are also observed (68,69,70). These changes resolve spontaneously and liver histology returns to normal without the development of fibrosis in patients who recover from this insult.

Passive Hepatic Congestion

Passive hepatic congestion occurs in patients with isolated right-sided heart failure or cardiopulmonary

disease associated with a persistent elevation in right atrial pressure (2,4,71). This may include biventricular heart failure secondary to alcoholic or ischemic dilated cardiomyopathy, isolated right coronary artery occlusion and right ventricular myocardial infarction, severe pulmonary hypertension, cor pulmonale, mitral stenosis, and various forms of congenital heart disease. Passive hepatic congestion may also develop in patients with hypertensive restrictive cardiomyopathy and end-stage renal failure. All patients with passive hepatic congestion have elevated central venous pressure, which is transmitted backwards through the hepatic veins, to the central veins of the hepatic acinus, and into the sinusoids of the hepatic lobule. Chronic liver injury resulting from this process is referred to as *congestive hepatopathy*. Over time this process may lead to cardiac fibrosis or cardiac cirrhosis.





▪ **Figure 41.6** Gross appearance of the cut surface of the liver in a patient with passive hepatic congestion. The characteristic appearance is of a "nutmeg" liver, small reddish areas surrounded by paler areas.

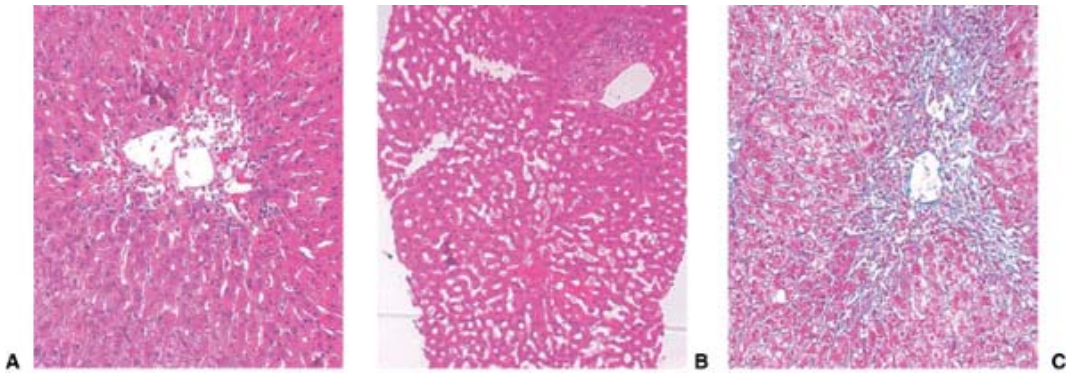
Pathophysiology

Evidence of congestive hepatopathy can be seen on gross pathologic examination of the liver. The liver is enlarged and appears congested and purplish with prominent hepatic veins. The cut surface of the liver reveals the classic description of "nutmeg liver" (Fig. 41.6); reddish central areas correspond to central vein congestion and hemorrhage into zone 3 of the hepatic lobule, surrounded by pale or yellowish areas representing zones 1 and 2 that are either histologically normal or contain fatty change. Microscopic examination reveals prominent central veins, central vein hemorrhage, dilated sinusoids, and hemorrhagic necrosis within zone 3 (2,72). Variable degrees of fibrosis may be seen circumscribing central veins (Fig. 41.7). Over time bridging fibrosis may extend between central veins, and this will eventually progress to form cirrhotic nodules. Histologically, this form of cirrhosis is unique compared to that resulting from all other forms of chronic liver disease in which portal-to-portal fibrosis occurs. In some cases, regeneration of periportal hepatocytes within zone 1 may yield discrete nodules lacking surrounding fibrosis. Such nodules do not represent cirrhosis and are referred to as regenerative nodular hyperplasia (see Chapter 42).

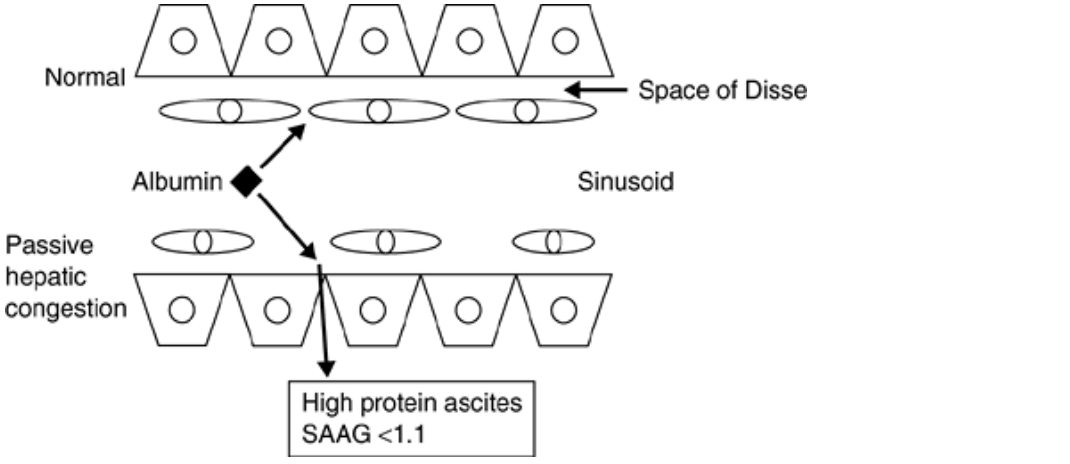
Chronic passive congestion of the hepatic lobule leads to a series of pathophysiologic alterations that causes cardiac cirrhosis and ascites formation and that renders zone 3 hepatocytes more susceptible to ischemic injury (72). When exposed to high pressure, as occurs with passive congestion, sinusoidal fenestrae enlarge and this allows large amounts of protein-rich fluid to enter the space of Disse (1). Accumulation of fluid within the space of Disse is typically drained by hepatic lymphatics. But when the capacity to drain hepatic lymph is

exceeded by the hemodynamic forces associated with passive congestion, high protein–containing fluid within the space of Disse leaks into the peritoneal cavity and produces high-protein ascites (Fig. 41.8) (4,73). Chronic passive congestion also leads to sinusoidal fibrosis that impairs the diffusion of nutrients and oxygen

across the space of Disse and into zone 3 hepatocytes, and it is this process that appears to increase the susceptibility of zone 3 hepatocytes to hypoxic injury in patients with coexistent left ventricular cardiac dysfunction. There appears to be no correlation between the elevation in right atrial pressure and the severity of liver injury in patients with passive hepatic congestion (2). In addition, zone 3 necrosis is rarely, if ever, seen in patients with isolated right ventricular failure in the absence of left ventricular failure and hypotension (3,24,28). It is therefore apparent that although patients with passive hepatic congestion may develop progressive fibrosis, cirrhosis, and ascites, hepatic function in patients with passive congestion is generally well preserved and liver-related mortality is rare except in the setting of coexistent left ventricular failure, hypotension, and hepatic ischemia.



▪ **Figure 41.7** Microscopic appearance of the liver with passive hepatic congestion. **A:** Congestion, dilatation, and hemorrhage at the central vein. **B:** Dilatation of sinusoids radiating outward from the central vein and sinusoidal fibrosis. **C:** Fibrosis of the central vein with extension of fibrosis radiating outward along hepatic sinusoids.



▪ **Figure 41.8** Schematic drawing of the pathophysiologic mechanisms

involved in the formation of ascites in patients with passive hepatic congestion. The normal hepatic sinusoid (*top half*) is lined with sinusoidal epithelium containing small fenestrae. In patients with passive hepatic congestion (*lower half*) the elevated hepatic venous pressure causes the sinusoidal epithelial fenestrae to increase in size. Large amounts of albumin and other plasma proteins enter the space of Disse, pass through hepatocyte junctions, and form ascites containing a high concentration of protein and a low serum to ascites albumin gradient (SAAG).

Clinical Features and Outcome

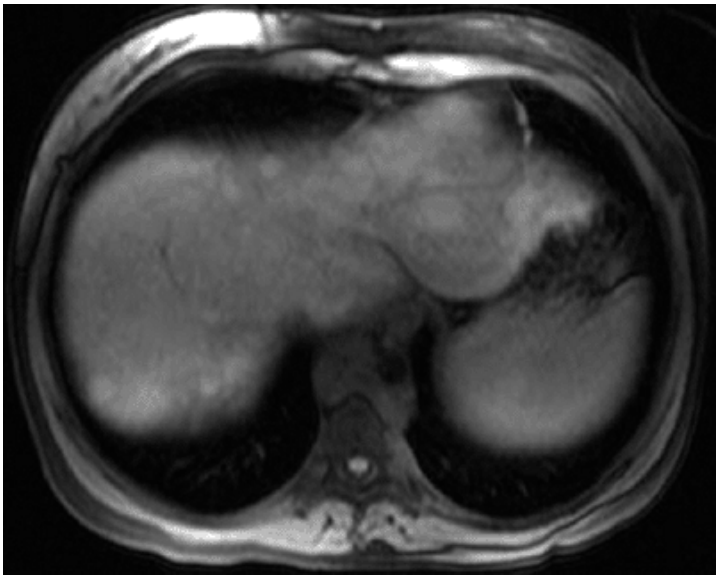
Patients with passive hepatic congestion are typically identified in one of three ways: They are asymptomatic and are found to have mild abnormalities in serum liver chemistries, a mild elevation in total bilirubin, or a mild prolongation in the INR; they present with symptoms of hepatic congestion, usually a dull ache in the right upper quadrant; or they present for evaluation of ascites. At the time of presentation, patients may already be known to have right ventricular failure or the accompanying cardiac disease may not have been appreciated. Regardless of the presentation, physical findings of right ventricular failure and passive hepatic congestion can be found. The most common of these include jugular venous distension, hepatomegaly, and hepatojugular reflux. In patients with marked tricuspid insufficiency, presystolic pulsations may be felt through the liver (74). Lower extremity edema may be absent in patients with isolated right ventricular failure but is typically present in patients with biventricular failure.

Laboratory studies may demonstrate mild elevations in the levels of serum liver AT, alkaline phosphatase, and bilirubin, although each of these chemistries may also be within the limits of normal. When an elevation in total bilirubin level is present, it is frequently the unconjugated fraction (4). In addition, the increase in serum bilirubin level appears to correlate with the severity of passive congestion and the elevation in right atrial pressure (2). In some patients with severe acute right ventricular failure and marked elevations in right atrial pressure the total bilirubin level may approach 20 mg/dL. Serum albumin level is typically normal or only slightly reduced. The most consistent abnormal laboratory value in patients with passive hepatic congestion is the INR, which is almost always prolonged to approximately 1.5. Serum ammonia level may be elevated in some patients but is only rarely associated with symptoms of hepatic encephalopathy (75). In patients with biventricular failure, episodes of hypoperfusion and hypoxia secondary to exacerbations of left ventricular failure may lead to more severe or abrupt abnormalities in serum liver chemistries.

Echocardiography findings are frequently abnormal and may demonstrate right ventricular contraction defect, tricuspid insufficiency, and/or an elevation in pulmonary arterial pressure. Hepatic ultrasound demonstrates hepatomegaly and diffuse increased echogenicity throughout the liver. Reversal of flow in the hepatic veins may be seen with Doppler ultrasonography. Contrast-enhanced CT scan and MR imaging demonstrate a heterogeneous appearance of the liver parenchyma (76). This is consistent with the gross “nutmeg” appearance of the liver that results from intervening areas of central vein hemorrhage and preserved portal blood flow (Fig. 41.9). Enlargement of the hepatic veins may also be present.

Ascites is a frequent finding in patients with passive hepatic congestion and this typically occurs in the absence of cirrhosis. The ascitic fluid contains a high concentration of protein, typically greater than 2.5 g/dL with a serum to ascites albumin gradient (SAAG) of less than 1.1 (77). When cardiac cirrhosis has developed from long-standing passive hepatic congestion, the SAAG usually remains below 1.1 but may approach or exceed this value, particularly in patients in whom a

coexistent underlying liver disorder has contributed to the development of cirrhosis. It is therefore helpful to assess liver histology in a patient with passive hepatic congestion, even in those with a low SAAG. Because it is difficult to perform percutaneous liver biopsy in most patients with ascites, a biopsy may need to be performed through the transjugular approach. In such patients, measuring hepatic venous pressure and calculating the hepatic venous pressure gradient (HVPG) is an excellent complementary way to help determine whether ascites is secondary to chronic passive congestion or is caused by a secondary liver disorder that has led to the development of cirrhosis. In patients with passive hepatic congestion both the free and wedged hepatic venous pressures are elevated and the HVPG is normal. This may also be true for patients with cardiac cirrhosis. In contrast, patients with passive congestion and cirrhosis, but who develop cirrhosis from another underlying chronic liver disease (i.e., chronic viral or nonviral hepatitis), will not only have an elevated free hepatic venous pressure but also an elevated HVPG.



▪ **Figure 41.9** Magnetic resonance image of a patient with severe pulmonary hypertension, right ventricular failure, tricuspid regurgitation, and jugular venous distension. Note the mottled appearance of the liver parenchyma that corresponds histologically to centrilobular areas of passive congestion with intervening areas of preserved portal blood flow.

Patients with passive hepatic congestion typically have stable hepatic function for prolonged periods, even with the presence of ascites and even after they have developed cardiac cirrhosis (2). Esophageal varices are rarely present even in

patients who have developed cardiac cirrhosis. The long-term mortality associated with passive hepatic congestion is therefore dictated by the mortality associated with the underlying cardiac disease; this is considerably reduced in patients with biventricular failure. Acute liver failure and liver-related mortality is only observed in those patients with biventricular failure in whom episodes of left ventricular failure lead to hypotension and a superimposed ischemic hepatitis (21,27,78).

Treatment

The treatment for passive hepatic congestion is to improve forward cardiac output. This is associated with an improvement in serum liver chemistries and a reduction in ascites formation. Ascites should be treated with diuretics. However, excessive use of diuretics could lead to dehydration, hypotension, and hepatic ischemia (78). Paracentesis should not be performed repeatedly as a treatment for refractory ascites in patients with passive hepatic congestion because this leads to severe protein loss and accentuates protein malnutrition owing to the high protein content of the ascitic fluid. Placement of a TIPS is contraindicated in the treatment for refractory ascites in this setting (79). This stent would provide a direct communication between the high-pressure central venous system and the portal system, lead to a marked increase in portal hypertension, and may precipitate massive variceal hemorrhage. Placement of a peritoneal–venous shunt is also contraindicated. In the setting of cardiac failure the delivery of additional volume to the heart is likely to exacerbate the underlying cardiac disease. In addition, the high pressure within the jugular vein of these patients will typically prevent flow of ascites from the lower-pressure abdominal cavity. Finally, the protein content of the ascitic fluid in patients with passive congestion tends to occlude the peritoneal–venous shunt within a relatively short period.

Several medications should be avoided or used with extreme caution in patients with passive hepatic congestion. These patients are extremely sensitive to warfarin (Coumadin); they have a baseline prolongation of INR, and this increases substantially when even very low doses of warfarin (Coumadin) are administered. Furthermore, acute exacerbations in cardiac failure will result in a marked prolongation in INR even in patients in whom this was previously well controlled. Patients with hepatic congestion also metabolize drugs more slowly. As a result, these patients are susceptible to toxicity from any drug that is metabolized by the liver and in cases in which an elevation in the blood level of the native drug may cause side effects.

Constrictive Pericarditis

Constrictive pericarditis produces clinical and pathologic changes similar to those observed in the Budd-Chiari syndrome (see Chapter 40). Indeed, it has been suggested that the original patient described by Budd actually had constrictive pericarditis as opposed to hepatic vein thrombosis (80). Constrictive pericarditis is

associated with marked elevations in central venous pressure that are sufficient to cause both zone 3 hemorrhage and necrosis. Cirrhosis develops much more rapidly in patients with constrictive pericarditis than in other forms of passive congestion. The physical examination is characterized by marked jugular venous distension that arises during inspiration (Kussmaul's sign), a pericardial knock, massive hepatomegaly, massive ascites, and peripheral edema. Liver AT levels

are variably elevated but jaundice is frequently absent (80,81). All signs and symptoms resolve after pericardiectomy.

Congenital Heart Disease

Congenital heart disease represents a wide spectrum of cardiopulmonary abnormalities that most commonly become apparent shortly after birth or within the first few years of life. Both passive hepatic congestion and/or hepatic ischemia can be seen in these children, the severity of which is determined by the particular cardiac anomaly present. As in adults, children with biventricular failure and both passive hepatic congestion and a low cardiac output state associated with hypotension and intermittent ischemic hepatitis develop the most severe liver injury and have the highest mortality. This is most commonly observed in children with hypoplastic left ventricle, coarctation of the aorta, and transposition of the great vessels (82,83,84). Major advances in the surgical treatment of congenital heart disease have significantly reduced early mortality in these infants and children. As a result, many now reach adulthood and develop side effects related to long-standing passive hepatic congestion (85).

Patients with passive hepatic congestion secondary to congenital heart disease present in a similar manner as other patients with this hepatic disorder. They are found to have abnormal liver chemistries or ascites. Their evaluation and management is likewise similar. However, many of these patients may also be found to have concomitant hepatitis C virus (HCV) infection from blood products they received at the time of cardiac surgery (86). In this situation, the underlying heart disease should not be considered an absolute contraindication for treatment of chronic HCV if indicated. As patients with congenital heart disease grow and reach adulthood, some may develop worsening heart failure and require cardiac transplantation (87). Passive congestion even with cardiac ascites should not be considered a contraindication to proceeding with cardiac transplantation as long as cirrhosis is absent. A complete evaluation of liver function, histology, and hemodynamics, to include the HVPG, should therefore be undertaken in patients with congenital heart disease who are being considered candidates for cardiac transplantation.

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Chapter 42
Benign Solid Tumors

Arie Regev

Key Concepts

- Benign solid lesions of the liver are detected with increasing incidence due to the frequent use of imaging studies of the abdomen. They are usually found incidentally in patients with no liver disease.
- Hemangioma of the liver is the second most common benign focal hepatic lesion (simple cyst being the most common one) and the most common *solid* hepatic tumor (1% to 7.4% of the normal population). It is rarely of significant clinical consequence.
- Focal nodular hyperplasia (FNH) is less common than hemangioma (0.4% of the normal population) and only rarely poses any significant risk.
- There is rarely an indication for a surgical intervention in patients with a hemangioma, FNH, or most other benign solid lesions.
- In contrast to hemangioma and FNH, hepatic adenoma is a rare tumor (0.004% of the normal population), which may cause significant morbidity from bleeding, rupture, or malignant transformation, and usually requires surgical resection.
- There is a strong association between hepatic adenoma and oral contraceptives (OCPs), and a history of prolonged use of OCPs should be sought in every woman with a focal hepatic lesion.
- A strong relationship to OCPs has not been unequivocally shown for hemangioma or FNH, although this issue remains controversial.
- A focal lesion in a patient with cirrhosis should not be regarded as benign and should be considered a malignancy until proved otherwise.

Benign focal lesions of the liver are found with increasing incidence because of the frequent use of imaging studies of the abdomen. They represent a relatively common reason for referral to the hepatologist or gastroenterologist. Most benign lesions are detected incidentally by imaging studies performed for unrelated reasons. Technical advances in imaging modalities have led to the identification of smaller lesions that until recently were not detected. Many of the lesions that present as a focal liver mass are true neoplasms, while others result from reactive proliferation of different cells. In general, benign tumors of the liver may arise from hepatocytes, bile duct epithelium, the supporting mesenchymal tissue, or a combination of two or more of these (Table 42.1).

Although most patients with benign hepatic tumors are asymptomatic, a minority may present with symptoms that may be local or systemic. In these patients, the relationship between the symptoms and the hepatic lesions may be difficult to

correlate, and additional workup is necessary to rule out other causes for the patients' complaints. In most cases patients with benign hepatic lesions have no preexisting liver disease, and the finding of a coexisting chronic liver disease such

as chronic hepatitis B, hemochromatosis, or cirrhosis should raise a suspicion for a malignant tumor. A conclusive diagnosis of a focal hepatic lesion is essential because it may represent a primary or secondary malignancy, which may require immediate treatment. In addition, some benign lesions carry specific risks such as rupture, bleeding, malignant transformation, consumptive coagulopathy, and disseminated intravascular coagulation. Focal solid hepatic lesions often represent a diagnostic challenge for the clinician and frequently mandate extensive evaluation. They may be difficult to characterize and at times impossible to differentiate from malignant tumors by the clinical presentation. Nevertheless, the importance of a detailed history and physical examination in the assessment of a patient with a newly discovered focal liver lesion cannot be overemphasized. Often the clinical presentation provides important clues to a specific diagnosis and suggests whether the tumor is benign or malignant. Still, in many cases the clinical information and the first imaging study are nondiagnostic and the clinician must choose additional studies from an ever-increasing number of available options. Despite continuing advancement in sensitivity and accuracy, imaging studies fail to yield conclusive diagnosis in a sizable number of patients, and in these cases histopathologic assessment is necessary to characterize the lesion. A tissue sample may be obtained by an ultrasonography or computed tomography (CT)-guided percutaneous liver biopsy or by a laparoscopic liver biopsy. Histopathologic evaluation remains essential in the clinical management of a significant number of tumors or masses in the liver; possible exceptions include hemangioma, focal nodular hyperplasia (FNH), and focal fatty change, which may be unequivocally diagnosed by imaging studies. Unfortunately, in some benign lesions (e.g., hemangioma and hepatic adenoma) liver biopsy carries a high risk of bleeding and is therefore contraindicated. A fundamental knowledge of the various lesions and their characteristic features should help in the differential diagnosis.

Table 42.1. Benign Solid Tumors of the Liver

EPITHELIAL TUMORS
Hepatocellular adenoma
Bile duct adenoma
Biliary cystadenoma
MESENCHYMAL TUMORS
Hemangioma
Infantile hemangioendothelioma
Fibroma
Angiomyolipoma
Lipoma
Lymphangioma
Benign mesenchymoma
MIXED TUMORS
Teratoma
TUMOR-LIKE LESIONS
Focal nodular hyperplasia
Nodular regenerative hyperplasia
Mesenchymal hamartoma

Microhamartoma (von Meyenburg complex)
Inflammatory pseudotumor
Focal fatty change
Pseudolipoma
Macroregenerative nodule

Focal Nodular Hyperplasia

Epidemiology

FNH is a benign tumor-like lesion of unclear etiology. It is the second most common benign solid mass of the liver (after hepatic hemangioma), and its incidence has been reported between 0.31% and 0.6% (1,2). It is diagnosed predominantly in adult women but has also been reported in men (female-to-male ratio of approximately 8:1) and children. In most cases, it is an incidental finding on abdominal imaging or surgery performed for unrelated reasons. FNH is solitary in most cases and is located in the right lobe more often than in the left (3). Two or more lesions are encountered in approximately 20% of the patients and a distinct minority may have more than five lesions (3,4,5). The size may range from 1 mm to more than 20 cm; however, most (64%) are smaller than 5 cm in diameter (3). Although many of the patients may have a history of oral contraceptive (OCP) use (6), the association of FNH with OCPs is controversial. Some authors have suggested increased prevalence and increase in size in women who use OCPs (7), but most studies found no relationship between estrogens and the size or number of FNH (8). Although these are two distinct lesions, there are rare reports of FNH coexisting with hepatic adenoma (9,10).

Pathogenesis

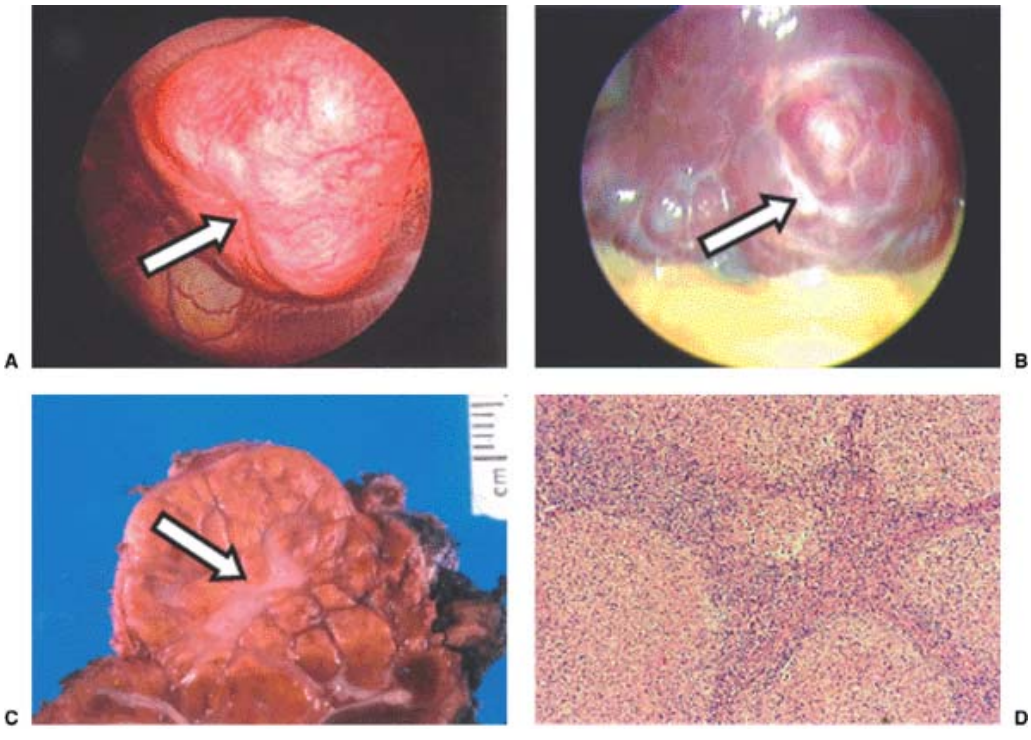
FNH is considered to be hyperplastic rather than neoplastic in origin, although its exact pathogenesis remains a matter of controversy. It typically occurs in the background of a healthy liver. It is believed by many to be a hyperplastic response to a preexisting vascular anomaly in the location of the lesion. According to this theory, the initial injury may be arterial malformation, which leads to increased arterial blood flow to a specific region compared to the adjacent parenchyma, which in turn leads to high sinusoidal pressure, resulting in hepatocellular hyperperfusion. This may be followed by local angiogenesis, leading to hyperplasia and causing the typical arterial branching in the center of the FNH (1). A recent report of FNH diagnosed in the same hepatic segment in identical twins supports the theory of a congenital anomaly being an initial event in the

pathogenesis of this lesion, at least in some patients (11). Because of the female predominance, a link with OCPs has been postulated; however, this relationship remains debatable. Some studies and case reports have suggested that the development of the vascular malformation or the growth of the FNH may be influenced by estrogen; however, this has never been unequivocally proved

(7,12,13). In contrast, other clinical studies have found no association between the use of OCP or pregnancy and FNH. One retrospective study found no such association in 216 women with FNH (8). Most FNHs are supplied by a single artery, which is typically enlarged. In contrast to the usual situation, this artery is not accompanied by a portal vein or a bile duct. In some cases, the FNH is predominantly supplied by the portal vein (13), which may result from thrombosis of the central artery in large lesions. Although some reports suggested that FNH may be a clonal lesion on the basis of a uniform pattern of X-chromosome inactivation (14,15), others have shown it to be of a clear polyclonal nature (16). Recently, several authors suggested a causal relationship between systemic cytotoxic chemotherapy (usually alkylating agents such as busulfan or melphalan) and subsequent development of FNH in adults and children (17,18). Injury to the intrahepatic vascular endothelium with ensuing localized circulatory disturbances was postulated as a possible mechanism.

Pathology

The typical macroscopic appearance is of a firm, nodular, mass with a dense central stellate scar and radiating fibrous septa that divide the lesion into lobules of various size (6,19,20) (Fig. 42.1A–C). The lesion is sharply demarcated from the surrounding liver tissue but has no true capsule. The central scar is clearly seen macroscopically in approximately 50% of the cases (3); however, in some of the cases it is absent and the fibrous septa may be poorly developed. The lesion is light brown or yellowish gray and usually occupies a superficial position. The average size is 5 cm and the lesion uncommonly exceeds 10 cm in diameter. Occasionally, it is exophytic or pedunculated. Larger lesions may have foci of hemorrhage or necrosis.



▪ **Figure 42.1** Focal nodular hyperplasia (FNH). **A** and **B:** Laparoscopic appearance of FNH. Both images show protruding lesions with central depressions *(arrows)* corresponding to central scars. **C:** Cut surface of a resected specimen of FNH showing a central scar *(arrow)* and cirrhosis-like appearance. **D:** Low-power view of an FNH shows a central fibrous scar, with

fibrous septa forming nodule-like structures.

Microscopically, FNH closely resembles the cirrhotic liver because the fibrous septa create a picture that is similar to that of regenerative nodules (Fig. 42.1D). The differentiation may be difficult in the absence of clinical information. However, a confident diagnosis of FNH can be made with a needle biopsy if the sample is known to come from a mass. The hepatic parenchyma between the septa may include all the components of normal liver including cords of hepatocytes, sinusoids, and Kupffer cells; however, it often lacks the normal liver architecture and may be devoid of central veins and portal tracts (6,20,21,22). Features of chronic cholestasis with accumulation of copper (demonstrated by rhodanine stain) and copper-binding protein (demonstrated by Victoria blue stain) are common (22). The Kupffer cells are usually active and show normal uptake of technetium-99m (^{99m}Tc) sulfur colloid, which is different from most cases of hepatic adenomas. Characteristically, the fibrous septa contain numerous bile ductules and blood vessels, as well as chronic inflammatory cells that may form a dense infiltrate. Branches of the hepatic artery and portal vein may show intimal and smooth muscle hyperplasia with thickening of their walls (6,20,21,22).

Telangiectatic FNH is considered an atypical variant of FNH in which the central scar is replaced by a telangiectatic lesion with radiating septa (13,23,24). This rare subtype is more likely to be symptomatic as a result of hemorrhage and necrosis. Recently, it has been suggested that telangiectatic FNH may be closer to hepatic adenoma and should be referred to as *telangiectatic hepatocellular adenoma* (23). Another rare subtype is mixed adenomatous and hyperplastic FNH (3,9).

Clinical Manifestations and Natural History

In most of the cases, FNH is asymptomatic and detected incidentally on imaging studies performed for unrelated reasons or during surgery. Hepatic biochemical tests are typically normal and the serum level of α-fetoprotein is not elevated.

Abdominal discomfort may be the presenting symptom in a minority of patients (25,26). Abdominal pain is rare and should prompt an evaluation for other causes. Early reports have suggested that women taking OCPs were more likely to be symptomatic (27,28) but this has not been supported by reported in later publications. Some patients may present with hepatomegaly, abdominal mass, or abdominal tenderness but most (approximately 85%) will have normal findings in the physical examination (3). Sudden abdominal pain may be related to rupture or bleeding that is distinctly rare (29,30). The association of rupture with OCPs, although suggested by early studies, remains doubtful (27,29,30,31,32,33). Fibrolamellar hepatocellular carcinoma (HCC) may be mistaken for FNH (34) or may occur in the same liver (35). However, malignant transformation has not been unequivocally described in FNH, and there is no evidence that FNH it is a precursor of HCC or fibrolamellar carcinoma. This is in contrast to hepatic adenoma in which malignant transformation has been well documented.

The prognosis of patients with FNH is almost invariably excellent. Most patients remain asymptomatic and exhibit no significant changes in lesion size over time (36). Occasionally, FNH may regress (37,38) and only a small minority (<10%) may show some increase in size (38). Any significant increase in size should therefore prompt an evaluation for another diagnosis such as HCC or fibrolamellar carcinoma.

Imaging Studies

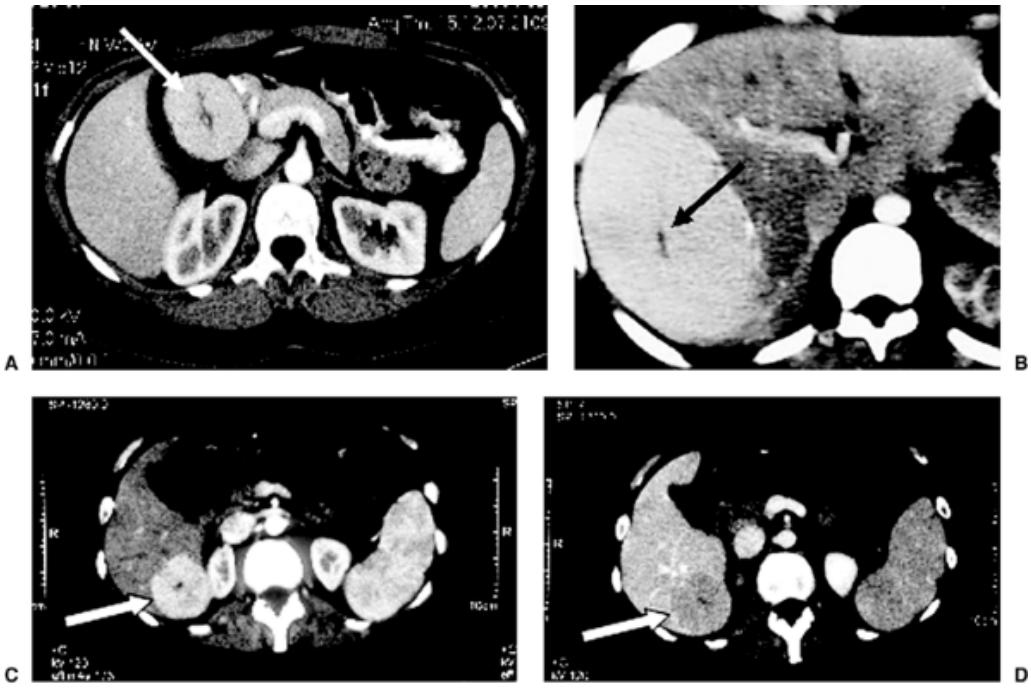
In most imaging studies, the typical appearance of FNH is related to the characteristic central scar, the hypervascularity, and the distinctive arterial blood supply, which begins in the center of the lesion with peripheral ramification in a spoke wheel pattern (39).

Standard ultrasonography is highly sensitive but nonspecific for the diagnosis of FNH. It usually demonstrates the lesion as a well-demarcated nodular mass but is frequently nondiagnostic (37,40). Ultrasonography detects the central scar in approximately 20% of the cases (41), although the addition of Doppler imaging may reveal arterial signals within the lesion in other cases (42). Power Doppler imaging has been reported to increase the rate of detection of the feeding artery from 22% to nearly 90% (43); however, arterial signal within the lesion may also be seen in malignant lesions. Ultrasonography–Doppler is therefore insufficient as a single modality to make the diagnosis of FNH.

Contrast-enhanced harmonic sonography (*CEHS*) has recently been demonstrated to show the typical spoke wheel pattern of the central arteries during the vascular phase in more than 90% of individuals with FNH (44). In addition, it produces a typical pronounced enhancement during the hepatic arterial phase and the early portal-venous phase that may help differentiate it from hepatic adenoma (45). This technique, which is based on the injection of a microbubble contrast agent, has been used for various focal liver lesions and may be a promising modality for the diagnosis of FNH.

CT scan should always be used in the multiphase contrast-enhanced mode for the evaluation of a possible FNH. The enhancement pattern may be helpful in suggesting the diagnosis or ruling it out (46,47,48). On precontrast images, FNH can be hypodense or isodense to the surrounding normal liver parenchyma. It is typically

hyperdense on arterial phase images (20 to 30 seconds after contrast injection) (Fig. 42.2A–C), becoming less prominent on portal-venous phase images (70 to 90 seconds) (Fig. 42.2D), and may become completely isodense (41,49). A central scar is seen in 43% to 60% of cases (50). It is typically hypodense on the precontrast and the arterial phase (Fig. 42.2A–C), becoming hyperdense on the portal-venous phase (Fig. 42.2D) (49). The scar may remain hyperattenuated on the delayed images because of slow washout of the contrast from the myxomatous tissue, but in contrast to hemangiomas FNH does not exhibit venous pooling (51,52). Occasionally, FNH may show imaging characteristics suggestive of a primary or metastatic malignancy. These may include the absence of a central scar, rapid washout, calcifications, or the presence of a capsule (53). Dynamic CT during hepatic arteriography has been shown to be helpful in such questionable situations because it clearly demonstrates the centrifugal blood supply through the fibrous stellate scar (54). However, CT arteriography has largely been replaced by advanced noninvasive imaging methods. The use of three-dimensional volume-rendered CT angiography (CTA) may demonstrate the characteristic anomalous feeding artery and may be helpful in distinguishing FNH from other lesions (55). Calcifications have been reported in 1.4% of 295 patients with FNH (34). This finding raises the suspicion of fibrolamellar carcinoma and usually mandates a surgical exploration to rule out this diagnosis.



▪ **Figure 42.2** Focal nodular hyperplasia (FNH). **A:** Arterial phase of a contrast-enhanced computed tomography (CT) scan shows an enhanced lesion protruding from the left hepatic lobe, with a nonenhancing central scar *(arrow)*. **B:** Arterial phase of a contrast-enhanced CT scan shows an enhanced lesion in the right hepatic lobe (segments 5 and 6), with a nonenhancing central scar *(arrow)*. **C:** Arterial phase showing enhanced lesion in segment 6 *(arrow)* with a central scar. **D:** Portal-venous phase shows the same lesion *(arrow)*, which is now mildly hypodense, but still showing the central scar.

Magnetic resonance imaging (*MRI*) usually shows FNH as a homogenous lesion that is isointense to mildly hypointense on T1-weighted images and isointense to slightly hyperintense on T2-weighted images. The margins are usually not well defined and the central scar may be identified by T2-weighted images in approximately 50% of the cases (49,52,56,57,58). On gadolinium-enhanced imaging, the lesion shows an early homogenous enhancement in the arterial phase and becomes isointense or slightly hyperintense compared to the surrounding liver in the venous and delayed phases. The central scar shows slowly progressive enhancement, which becomes maximal on delayed images. This characteristic appearance may differentiate FNH from malignant hypervascular tumors (59) and has a sensitivity of 70% to 80% and specificity of

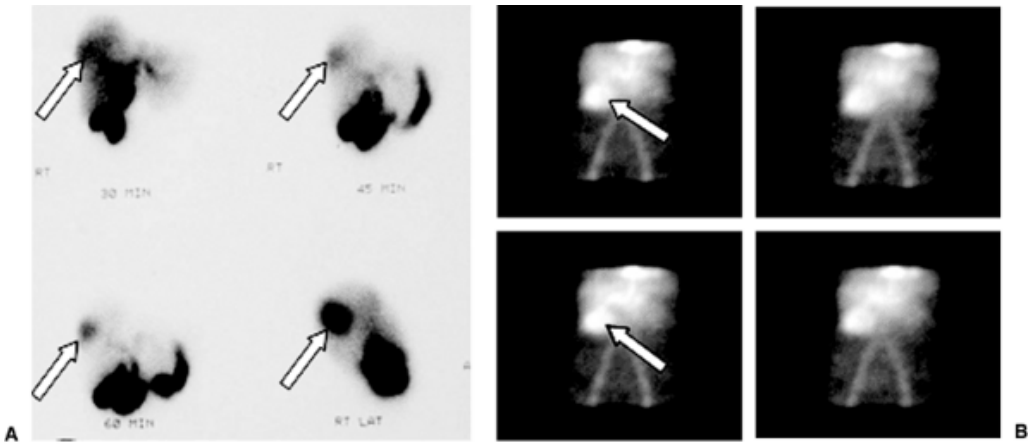
98% in lesions that are larger than 2 cm in size. Another distinguishing feature on gadolinium-enhanced images is the lack of capsule enhancement that is commonly seen in adenomas and HCCs. Small FNH (<1 to 2 cm) may appear more uniform in enhancement and the central scar may not be perceived.

Despite the distinguishing features, fibrolamellar carcinoma may look similar to FNH on imaging studies. Fibrolamellar carcinoma is typically larger, usually over 10 cm, at presentation. Its central scar frequently shows lower intensity than the surrounding tumor on T2-weighted images (in contrast to the higher signal produced by the central scar in FNH), with radiating enhancing bands on postgadolinium

images. Three-dimensional gadolinium-enhanced MR angiography (MRA) may increase the accuracy of the test for the diagnosis of FNH by demonstrating the characteristic vessels radiating from the center to the periphery of the lesion (the so-called star sign) (60). The use of liver-specific MRI contrast agents (i.e., reticuloendothelial and hepatobiliary agents) offers greater lesion-to-liver contrast than the conventional extracellular contrast agent, gadolinium chelate, which has a nonspecific distribution (57,61). These agents may be helpful in a small fraction of cases that remain ambiguous. Administration of superparamagnetic iron oxide (SPIO) particles, which undergo phagocytosis by Kupffer cells, may help distinguish benign from malignant lesions (62,63,64,65). Serial use of different liver-specific contrast agents such as gadolinium 1,4,7,10-tetraaza-dodecane-1,4,7,10-tetraacetic acid (Gd-DOTA) followed by SPIO and gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) may further increase the specificity of MRI for FNH (66). Unlike SPIO, Gd-EOB-DTPA is taken up by the hepatocytes and is excreted through the biliary system (67). Recently, the use of the hepatocyte-selective contrast agent gadobenate-dimeglumine (Gd-BOPTA) has been shown to improve the differentiation between FNH and adenoma with a sensitivity of 80% and specificity of more than 96% (68,69).

^{99m}*Tc sulfur colloid scintigraphy* is currently used less commonly by most centers for the diagnosis of FNH (49,61). FNH takes up ^{99m}Tc sulfur colloid at the same or greater rate compared to the surrounding liver parenchyma in 50% to 70% of patients. Hyperconcentration of the colloid by the lesion (Fig. 42.3A) is uncommon (approximately 7%) but is considered typical of FNH because of the highly active Kupffer cells within the lesion (70,71). Nevertheless, the reliability of this modality is poor in lesions smaller than 3 to 4 cm in size. Single photon emission computed tomography (SPECT) has a greater sensitivity compared with planar scintigraphy (72); however, lesions smaller than 2 cm in size are detected in only 11% to 45% of the cases (73). Scintigraphy with specific agents has been shown to improve the specificity of the test. The hepatocytes receptor ligand ^{99m}Tc-galactosyl-neoglycoalbumin (^{99m}TcNGA) has shown normal or increased uptake by FNH compared to the normal liver and, with concurrent use of SPECT, was useful in the differential diagnosis of FNH and malignant hepatic tumors (74). Scintigraphy with ^{99m}Tc-labeled derivatives of iminodiacetic acid (IDA) may also show a characteristic hyperconcentration (73). Combinations of scintigraphy, ultrasonography, and

contrast-enhanced CT scan or MRI have yielded a sensitivity of 82% to 90% and a specificity of 90% to 97% for the diagnosis of FNH (38,75,76).



▪ **Figure 42.3** Focal nodular hyperplasia (FNH) and hepatic hemangioma. **A:**

FNH—^{99m}Tc sulfur colloid liver scan demonstrates a focal lesion with enhanced uptake in the right hepatic lobe *(arrows)*. **B:** Hemangioma—tagged red blood cells (^{99m}Tc pertechnetate-labeled red blood cells [^{99m}Tc-RBC]) study in a patient with a single large hemangioma in the right hepatic lobe showing increased concentration of the isotope within the lesion *(arrows)*.

Positron emission tomography (PET) scan using ^{l8}F-fluorodeoxyglucose (^{18}F-FDG) has been shown to be helpful for the differentiation of FNH from liver metastases in patients with cancer (77). Whereas in malignant liver lesions the accumulation of ^{18}F-FDG is typically increased because of increased glucose metabolism, FNH lesions show normal or even decreased accumulation of ^{18}F-FDG (77,78). Although PET scan is not specific for FNH, it may be helpful when radiologic methods are not diagnostic.

Angiography is used infrequently because of the advances in noninvasive imaging methods. FNH typically appears as a highly vascular mass with an intense capillary blush (6,71). The central feeding artery is seen in approximately 60% of the cases. Hepatic arteriography has been used in combination with dynamic CT for the diagnosis of FNA. This combined technique may clearly demonstrate the characteristic centrifugal blood supply through the fibrous stellate scar even in small lesions (54). Occasionally, angiography is used before surgery as part of the preoperative evaluation.

Diagnosis

The diagnosis of FNH may be a complex and challenging process for the clinician.

The differential diagnosis includes numerous types of focal lesions, some of which may appear indistinguishable from FNH on initial workup (Tables 42.1 and 42.2). Malignant tumors such as HCC, fibrolamellar carcinoma, intrahepatic cholangiocarcinoma, and vascular hepatic metastases should be ruled out as early as possible (49). Typically, the patient with FNH will be asymptomatic and present with normal hepatic biochemical test results. Significant symptoms and any abnormality in the liver biochemistry should raise the suspicion of a neoplastic lesion. FNH is characteristically stable in size, whereas malignant lesions such as HCC may show growth on consecutive imaging studies.

Hypervascular metastases usually present in older individuals with a history of malignancy. They do not have a central scar and are typically hypodense

with a ring enhancement on the vascular phase of enhanced CT scan (79). HCC may show vascular invasion, lymphadenopathy, and metastases, which are all characteristic of a malignant lesion rather than FNH. HCC commonly shows a peripheral capsule on the delayed phases of contrast-enhanced CT scan or MRI (80); however, rim enhancement may occasionally be seen in FNH as well. In fibrolamellar carcinoma a central scar is seen in as many as 55% of cases, making the differential diagnosis from FNH extremely difficult. The appearance of calcifications should also alert the clinician to the possibility of fibrolamellar carcinoma rather than FNH. Central necrosis or hemorrhage may be seen in fibrolamellar carcinoma, hepatocellular adenoma, and HCC and may be confused with a central scar. MRI can usually assist in differentiating the central scar of FNH from necrosis or hemorrhage. The central scar typically appears hyperintense on T2-weighted images, whereas necrosis and hemorrhage appear hypointense. PET scan with ^{18}F-FDG may assist in the differential diagnosis from malignant lesions because FNH shows no increase in glucose metabolism, in contrast with many malignant tumors (77). Large

hemangiomas may have a central scar due to a previous hemorrhage or fibrosis. However, they exhibit a characteristic behavior in the early phases of contrast enhancement, as well as on delayed images on CT scan or MRI. The signal intensity of hemangioma on T2-weighted images may also assist in its differentiation from an FNH. Some hemangiomas may show calcifications.

Table 42.2. Imaging Features of Focal Nodular Hyperplasia, Hemangioma, and Hepatic Adenoma

	Focal nodular hyperplasia	Hemangioma	Adenoma
Ultrasonography	Usually nondiagnostic Variable echogenicity Occasionally central scar	Hyperechoic lesion with well-defined borders	Usually nondiagnostic
Doppler	Arterial flow within the lesion	No internal flow	Venous signals within the lesion (nondiagnostic)
Contrast-enhanced triple phase CT scan	Precontrast: Hypo- or isodense lesion Homogenous arterial enhancement with a hypodense central scar May turn isodense post contrast	Precontrast: Hypodense lesion Centripetal globular enhancement Retained contrast on delayed venous phase	Precontrast: Hypo- or isodense lesion Irregular enhancement with peripheral arterial enhancement postcontrast
MRI unenhanced	Low signal on T1 Slightly hyperintense on T2 Central scar hyperintense on T2	Well-circumscribed homogenous lesion Low signal on T1 Very high signal on T2	Low to slightly hyperintense area on T1 Well-defined low-intensity capsule Heterogeneous enhancement on T2
Gadolinium-enhanced MRI	Homogenous arterial enhancement Hypodense	Progressive centripetal enhancement Similar to CT	Enhancement as in CT scan

	central scar Contrast accumulates in central area on delayed T1	scan	
Angiography	Dilated hepatic artery Highly vascular lesion, with a central vascular supply Spoke wheel pattern in one third of the patients	Venous lakes with well-defined circular shape Displaced arterial branches Delayed venous phase	Hypervascular lesion—50% Hypovascular lesion—50% Peripheral vascular supply
Scintigraphy with ^{99m}Tc-labeled RBC	Equal or increased uptake in 50%–70% of the patients	Increased uptake by the lesion during venous phase Retention on delayed images	Hypoconcentration of the colloid (focal defect) in most patients

CT, computed tomography; MRI, magnetic resonance imaging; RBC, red blood cells.

Differentiating FNH from hepatic adenoma is important because their complications and management are different, but it may be difficult and occasionally impossible. Hepatic adenoma is strongly associated with the long-term use of OCPs. It tends to be larger (10 cm in size) and typically does not have a central scar. ^{99m}Tc sulfur colloid scintigraphy classically shows decreased uptake in most patients with adenoma compared to an uptake which is the same as or greater than that of the surrounding hepatic parenchyma, occurring in 50% to 70% of patients with FNH (Fig. 42.3A) (49,71).

Patients with atypical or equivocal lesions should undergo a CT scan or ultrasonography-guided needle biopsy to confirm the diagnosis. When imaging studies suggest a highly vascular lesion such as adenoma, hemangioma, or a vascular metastasis, a laparoscopic or surgical biopsy or resection should be performed to avoid the risk of uncontrolled bleeding associated with a percutaneous liver biopsy.

Management

Because the incidence of complications is extremely low, the recommended

treatment in asymptomatic FNH is observation (31). To ensure stability of the lesion size, it is recommended to repeat abdominal imaging 3, 6, 12, and 24 months after the diagnosis. If the lesion is highly suggestive of FNH and does not change over a period of 1 to 2 years, no further specific observation is indicated. In symptomatic patients, it is important to corroborate a causative relationship to the lesion and rule out other causes for the symptoms. If the lesion is enlarging or is clearly symptomatic, surgical intervention should be considered. It has been recommended that pedunculated lesion be resected to prevent the rare risk of torsion; however, there is scant evidence to support or oppose this recommendation. Although the role of OCPs remains controversial, there are a few reports of lesions that decreased in size after the discontinuation of OCPs (12,36,81). Discontinuation of OCPs should probably be considered in a large or symptomatic FNH; however, there is not enough convincing data to support early termination of pregnancy in a patient with a newly diagnosed FNH or preventive resection of FNH when pregnancy is contemplated.

When surgery is indicated wedge resection and enucleation are generally the recommended approaches (12,38,81). In many cases, they can be performed laparoscopically with excellent results because the lesions tend to be smaller and peripheral (82). In patients with a large FNH, a segmental resection or a formal lobectomy may be required. Angiographic embolization and hepatic artery ligation are alternative approaches for unresectable lesions (24,36,83,84).

Hemangioma

Epidemiology

Hemangioma is the most common benign *solid* lesion of the liver. The reported prevalence at autopsy ranges from 0.4% to 7.4% (19,21). Among the benign focal hepatic lesions, it is second in prevalence only to simple cysts. Hemangiomas are most often found incidentally and have no major clinical implications. They are more prevalent in women and in the right hepatic lobe; the sex ratio is between 4:1 and 6:1. Hemangiomas may present at any age but are most common between the third and fifth decades and rare in young children. There is some controversy about the term *cavernous hemangioma*. Whereas some authors use it as a general name for hemangiomas, others use it to describe a stage of development of the lesion (20).

Pathogenesis

Hepatic hemangiomas are congenital vascular malformations. They enlarge by ectasia rather than hyperplasia or hypertrophy and are considered hamartomas by

most investigators (85). They compress, rather than infiltrate, the surrounding liver parenchyma, which results in a dissectible plane between the hemangioma and liver tissue. There is an ongoing controversy about the role of estrogen in their growth. Although most researchers found no association (86), some have reported growth or initial symptoms during pregnancy or in women receiving OCPs (87,88,89). These reports suggest that although hemangiomas are unrelated to estrogens in most women, they may play a role in individual cases.

Pathology

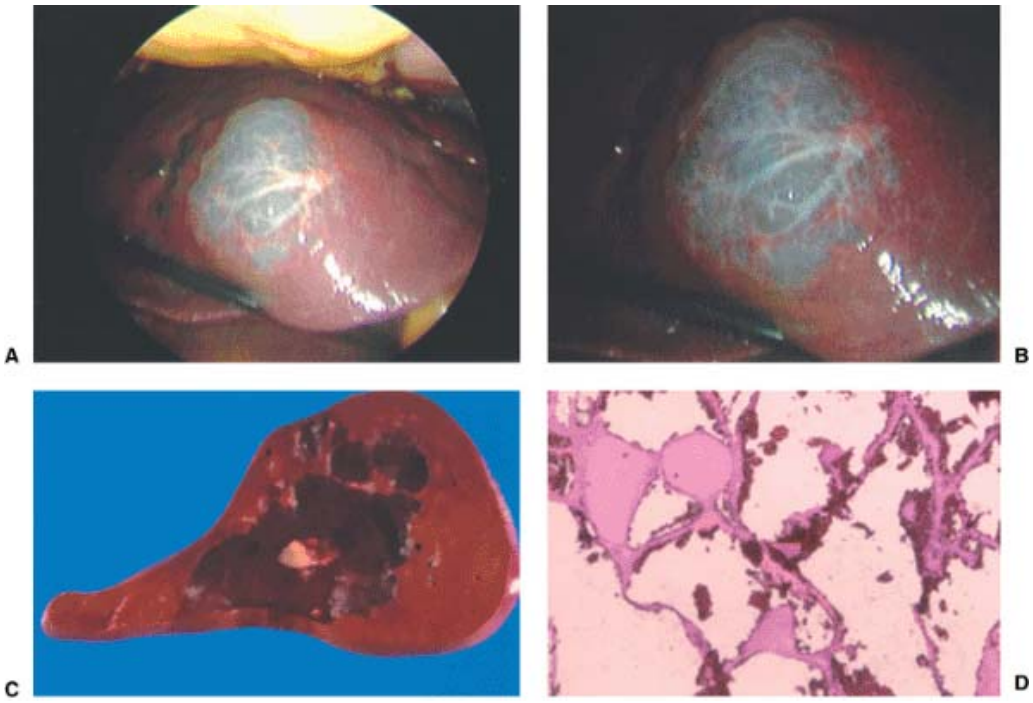
On gross examination hemangioma appears as a dark purple compressible spongy lesion (Fig. 42.4A,B) that may replace considerable portions of liver parenchyma (Fig. 42.4C). On microscopic examination, a hemangioma is typically composed of multiple vascular spaces of varying sizes, which are lined by a single layer of endothelial cells and are separated by fibrous septa (Fig. 42.4D). Intraluminal thrombi may be present and may lead to local calcifications. Nevertheless, biopsy is

usually unnecessary and may be associated with significant morbidity because of bleeding.

Clinical Manifestations

In most cases, hemangiomas are small and asymptomatic. Infrequently, they may grow to a large size, causing pressure or displacement of adjacent structures. Hemangiomas larger than 4 cm in size have been referred to as *giant hemangiomas* (90,91). Large hemangiomas are uncommon; however, they are more likely to cause symptoms. The most common complaints are of abdominal pain or discomfort; however, early satiety, nausea, and vomiting may also occur (90,92,93,94). Pain may be intermittent and is likely due to distension of the liver capsule or pressure on adjacent structures. Severe sudden pain may be caused by infarction, necrosis, or bleeding into the hemangioma. Other rare manifestations related to giant hemangiomas include gastric obstruction, obstructive jaundice (95), and symptoms resembling polymyalgia rheumatica (96). The relationship between symptoms and the hemangioma may be difficult to ascertain, and in many cases other

causes are discovered, such as peptic ulcer, gastroesophageal reflux disease (GERD), hernias of the abdominal wall, or tumors of the gastrointestinal tact. In one series of 87 patients with hepatic hemangiomas, 54% of patients were ultimately found to have other causes for their symptoms (97). In these cases, symptoms may resolve after treatment of the concomitant illness and therapy for the hemangioma is not required (93,97).



▪ **Figure 42.4** Hemangioma. **A** and **B:** Laparoscopic appearance of a giant hemangioma showing a well-circumscribed soft lesion with dark colored areas. **C:** A cut surface of a giant hemangioma occupying a large part of the right and left lobes. **D:** Histologic examination exhibits numerous dilated vascular channels with thickened, acellular fibrous septa. (Courtesy of P.A. Bejarano, MD, Department of Pathology, University of Miami School of Medicine.)

Physical examination findings are usually unremarkable. Occasionally, there is abdominal tenderness over the right upper quadrant and a palpable mass may be encountered. Rarely, a bruit may be heard over a large hemangioma.

Hepatic biochemical tests are usually normal and are therefore of little help in the diagnosis of hepatic hemangioma. On rare occasions, serum aminotransferases or alkaline phosphatase level may be mildly elevated. Serum levels of α-fetoprotein and carcinoembryogenic antigen (CEA) are invariably normal.

Complications and Natural History

Spontaneous bleeding into a hemangioma, although extremely rare, was reported by several authors (93). This complication typically presents with abdominal pain in the right upper quadrant and declining hematocrit, in the absence of trauma. The diagnosis is usually made on an abdominal CT scan and in some cases the treatment is removal of the hemangioma by lobectomy or enucleation. Thrombosis within a hemangioma may also present with right abdominal pain and occasionally with fever and increase of the erythrocyte sedimentation rate (95). Spontaneous rupture of hepatic hemangiomas is exceedingly rare (97,98); however, there are several reports of rupture of giant hemangiomas of the right lobe after abdominal trauma (99). A few patients presented with obstructive jaundice due to pressure on bile ducts (95,100) or portal hypertension due to pressure on the portal vein (101). Hemangiomas have rarely been reported to grow rapidly during pregnancy, and after the use of estrogens; however, the effect of pregnancy and estrogens on growth is

inconsistent (87,88,89,102). Rarely, patients with giant hemangiomas may develop consumption coagulopathy within the hemangioma and may present with evidence of disseminated intravascular coagulation (DIC), the so-called Kasabach-Merritt syndrome (87,93,103,104). The pathogenesis is likely platelet trapping in the hemangioma that leads to activation of the clotting cascade and consumption of both platelet and clotting factors.

Despite rare reports of complications, the long-term clinical course of most hepatic hemangiomas is benign (105,106), and most patients will never experience symptoms. When followed up for periods of 15 and 20 years, most patients remained asymptomatic and showed no significant changes in quality of life (85,98). In one of the largest series, 158 hemangiomas in 123 subjects were followed up for 12 to 60 months. No complications were observed during the follow-up period. Only one patient developed new symptoms, and only one hemangioma showed a significant change in size (107).

Imaging Studies

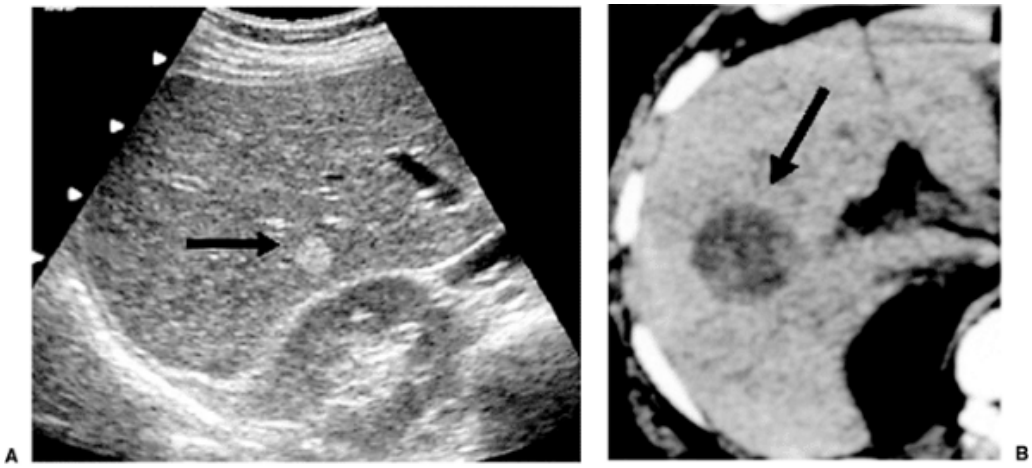
Plane abdominal radiographs are usually unhelpful for the detection or diagnosis of hemangiomas. Rarely they may show calcifications, which are more commonly seen in larger hemangiomas and elderly patients (108,109).

The typical *ultrasonographic* appearance of a hemangioma is of an echogenic, well-demarcated homogenous lesion with well-defined borders (Fig. 42.5A) (40). Ultrasonography can establish the diagnosis of a hemangioma in 80% of lesions smaller than 6 cm in size (97,110). However, approximately 20% to 30% of the patients may present with an atypical sonographic appearance (40). Posterior acoustic enhancement is a common feature. Doppler does not usually detect flow within the hemangioma because of the slow blood flow (111). Larger hemangiomas are more heterogenous and occasionally contain central fibrosis due to a previous hemorrhage. These usually require further imaging studies (112). Recent evidence suggest that contrast-enhanced harmonic ultrasonographic images using a microbubble contrast agent may be superior to conventional Doppler ultrasonography in differentiating hepatic hemangioma from HCC or hepatic metastases (109,113,114). A slow centripetal filling of the lesion by the contrast agent may be diagnostic of a hemangioma and may obviate the need for additional imaging studies.

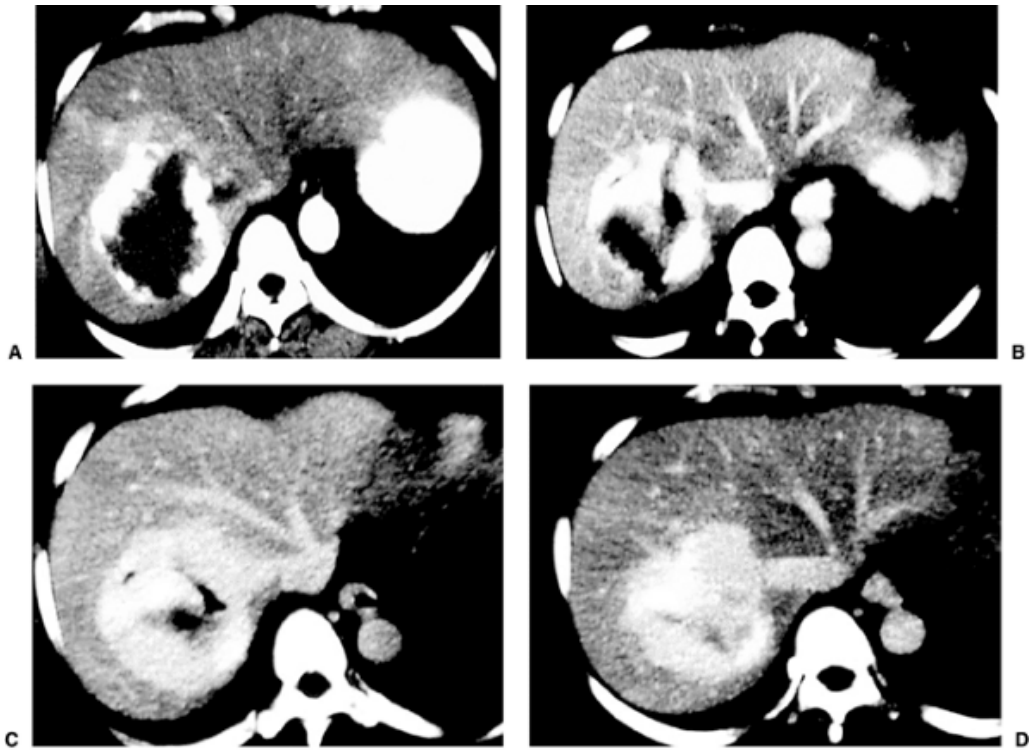
Dynamic CT scan offers a few advantages to ultrasonography in the diagnosis of hepatic hemangioma and is often performed to verify the diagnosis suggested by the ultrasonography. Almost all hepatic hemangiomas measuring 2 cm or more can be diagnosed by this technique. The hepatic triphasic CT scan technique is the most effective way to visualize a hemangioma. Before the administration of intravenous contrast, the typical appearance is that of a well-defined hypodense lesion (Fig. 42.5B). Serial images in the arterial phase of contrast administration show early peripheral enhancement with subsequent progressive globular centripetal filling (79,115,116) (Fig. 42.6A–D). Globular enhancement in the arterial phase represents venous lakes within the hemangioma and may be seen in 94% of large

lesions. It accurately differentiates between a hemangioma and a metastatic lesion (79,116,117). It usually takes about 3 minutes for complete opacification of the lesion to occur; however, it may take 10 to 15 minutes to complete opacification, and in many patients the center of the lesion may remain hypodense because of a central hemorrhage or fibrosis (118,119). Smaller lesions generally fill more rapidly and larger lesions are more likely to show slower central filling. Large hemangiomas (>4 cm) typically develop central areas that fail to fill in on the delayed enhanced

images. The center of the hemangioma is more likely to remain hypodense as the size increases. The contrast agent classically remains within the hemangioma for a long time (as long as 60 minutes) after the injection. This will not be demonstrated on standard images and the clinician should specifically ask for delayed images to obtain this typical picture. Occasionally, calcifications may be seen within a large hemangioma. The sensitivity and specificity of *dynamic CT scan* are more than 90% in lesions larger than 2 cm in size; however, they are significantly lower in smaller lesions (118,119,120,121).



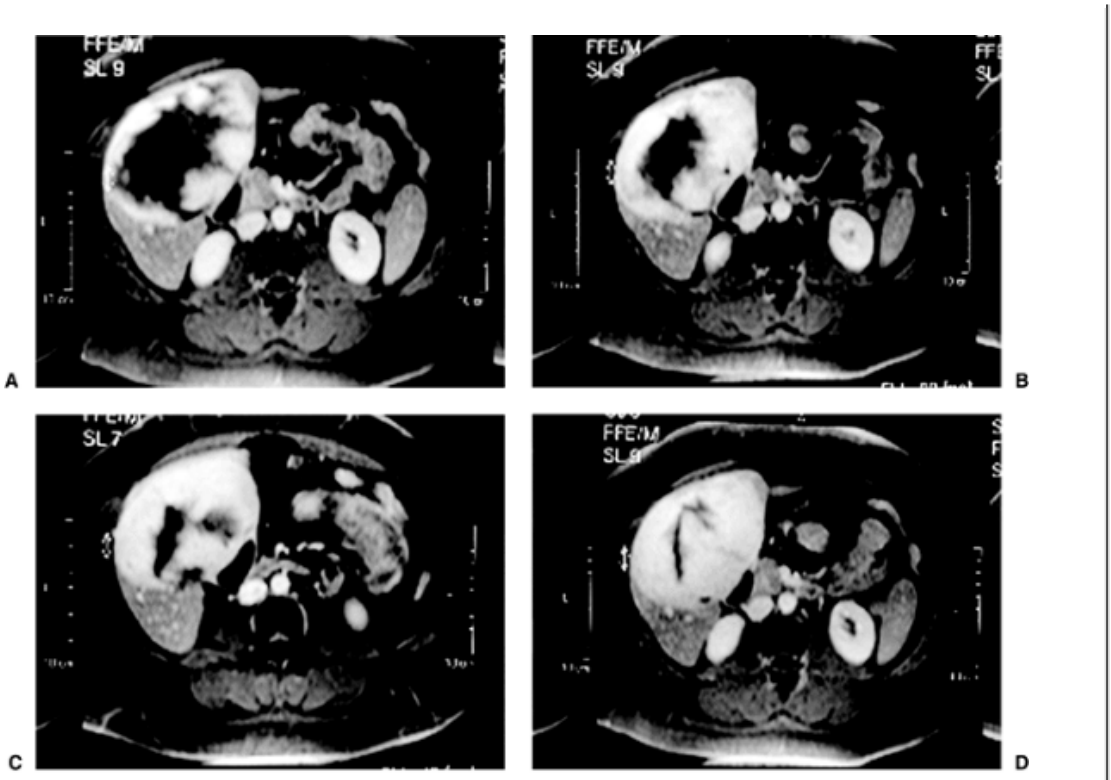
▪ **Figure 42.5** Hepatic hemangioma. **A:** Ultrasonography. A transverse view of the right hepatic lobe shows a well-circumscribed echogenic mass *(arrow)* that is consistent with a hemangioma. **B:** Computed tomography scan, precontrast injection, shows a well-circumscribed mass with low attenuation in the right hepatic lobe *(arrow)*.



▪ **Figure 42.6** Hepatic hemangioma. Dynamic, contrast-enhanced computed tomography (CT) scan demonstrates globular peripheral enhancement with gradual diffusion of the contrast from the periphery to the center of the lesion **(A)** 40 seconds, **(B)** 60 seconds, **(C)** 90 seconds, and **(D)** 120 seconds after intravenous contrast injection.

MRI shows a high degree of sensitivity and specificity (both >90%) in the diagnosis of hemangioma and is generally considered to be superior to CT scan for this purpose (57,122,123,124,125). It has a special value for the diagnosis of hemangiomas smaller than 2 cm in size and in patients with contraindications to the use of iodine-based intravenous contrast. A hemangioma usually appears as a well-circumscribed lesion that shows a moderately elevated signal on T2-weighted images and a low signal on T1-weighted images. The increased signal on T2-weighted images is typically less intense than that demonstrated by a simple cyst. Similar to the findings on CT scan, contrast enhancement with gadolinium shows a centripetal nodular filling on the arterial phase, which is considered by many to be a pathognomonic finding (Fig. 42.7A–D). Venous and delayed phases show progressive enlargement and coalescence of the peripheral nodules with variable degree of central filling (124,126). As in CT scan, larger hemangiomas commonly do not fill in on delayed enhanced images. They may show central cystic areas that are as bright as simple fluid, such as cerebral spinal fluid. Small (<1 cm) lesions may

fill quickly and may be difficult to delineate from other arterial phase-enhancing neoplasms such as small HCC or hypervascular metastases. In such cases, the distinguishing features can be found on venous and delayed images in which other vascular neoplasms commonly show rapid washout whereas hemangioma demonstrates persistent enhancement. Breath-hold images may improve the quality of the images and decrease motion artifacts (127).



▪ **Figure 42.7** Hepatic hemangioma. Contrast-enhanced magnetic resonance imaging (T1-weighted images) shows a low-signal lesion with peripheral globular enhancement on the arterial phase followed by progressive central filling—**(A)** 40 seconds, **(B)** 60 seconds, **(C)** 90 seconds, and **(D)** 120 seconds after intravenous gadolinium injection.

^{99m}Tc pertechnetate-labeled red blood cells (^{99m}Tc-RBC) pool scintigraphy may occasionally be helpful in controversial cases, but the use of this technique is decreasing in most medical centers. It typically shows initial hypoperfusion during the arterial flow phase followed by a gradual increase in the isotope in the lesion, which peaks at 30 to 50 minutes after the injection (Fig. 42.3B). Delayed images usually show retention of the tracer, which is typical of hemangioma. ^{99m}Tc-RBC scan has low sensitivity for lesions smaller than 2 cm in size. Furthermore, false-negative results may occur in the presence of fibrosis or thrombosis of the hemangioma. The sensitivity for lesions larger than 2 cm in size varies from 69% to 82% and the specificity approaches 100% (70). False-positive results are rare but well documented. Both HCC and angiosarcoma may rarely masquerade as a hemangioma on ^{99m}Tc-RBC scintigraphy (128), and the diagnosis should never be made solely on the basis of this study.

SPECT using ^{99m}Tc-RBC has been shown to increase the resolution of planar scintigraphy (75) and has a clear advantage over ultrasonography in differentiating large hepatic hemangiomas from other solid masses (129). The sensitivity and specificity of ^{99m}Tc-RBC SPECT may be as high as 90% to 97% in lesions larger than 2 cm in size, which is close to the accuracy of MRI (130,131), but the specificity may decrease to 50% in lesions with mixed echoic pattern (132). In patients with small lesions (<2 cm in size) MRI is considered more sensitive but less specific than SPECT; however, the use of dynamic three-view display of ^{99m}Tc-RBC SPECT improves its sensitivity in small lesions (between 1 and 2 cm in size) (133). Three-headed high-resolution SPECT shows a sensitivity of 100% for lesions of size 1.5 cm

or larger, but only 33% for lesions smaller than 1.5 cm in size (134,135). As in planar ^{99m}Tc-RBC scintigraphy, HCC may rarely mimic a hemangioma on ^{99m}Tc-RBC SPECT (136).

^{99m}Tc-RBC SPECT is best used to clarify a doubtful lesion on CT or to confirm a suspected hemangioma seen as a hyperechoic lesion on ultrasonography. Conventional *SPECT* with no ^{99m}Tc-RBC is less accurate for the diagnosis of hepatic hemangioma and may be of limited value for lesions less of 2.5 cm in size that are close to the heart or major intrahepatic vessels (137).

Catheter angiography is rarely used for the diagnosis of hepatic hemangioma, usually in uncertain or atypical cases in which other modalities have failed to yield a definitive diagnosis. Even in these cases, many centers prefer advanced imaging techniques such as CTA and MRA to standard catheter angiography. The contrast injected to the common hepatic artery rapidly fills the vascular spaces, creating a "starry night" appearance in the early phase and vascular lakes in later phases, which remain opacified beyond the venous phase (>40 seconds) (138). The borders may be irregular but are well defined, and the feeding vessels are typically displaced and curled at the margins of the lesion. Arteriovenous shunting is typically absent and its presence should raise suspicions of HCC. Still, hepatic hemangiomas may show atypical features mimicking HCC such as early fading after arterial enhancement (139,140), arteriovenous shunting (141), and centrifugal rather than centripetal enhancement pattern (142). Conversely, HCC may rarely mimic cavernous hemangioma on angiography (143). Discrepancy in different imaging studies should therefore warrant further follow-up and evaluation. A combination of ultrasonography, contrast-enhanced CT scan, and ^{99m}Tc-RBC scintigraphy was shown in one study to have a sensitivity of 86%, specificity of 100%, and accuracy of 91% in differentiating hepatic hemangioma from FNH and adenoma in 437 patients (38).

Management

Because most hemangiomas are asymptomatic, rarely enlarge in size or rupture, and have no malignant potential, surgical intervention is rarely indicated (25,97,144). When the diagnosis of hemangioma is conclusive, observation is sufficient and the rare risk of rupture should not be considered as an indication for surgery. Surgical therapy is indicated only in patients with severe symptoms, complications, or inconclusive diagnoses that cannot be resolved with imaging studies. Biopsy should be avoided because of high risk of bleeding (145). Overall, surgical resection is indicated in about 2% of diagnosed hemangiomas (97). This percentage may increase considerably in referral centers because of selection bias. The most common cause for surgical intervention in patients with hemangiomas is symptoms. The causative relationship between the hemangioma and symptoms should be confirmed and additional tests should be considered to rule out other potential causes such as gastroesophageal reflux disease, peptic ulcer, or gallstones. When symptoms are clearly related to the hemangioma, resolution may be observed in as many as 96% of the patients after surgical treatment (93). Another major reason for surgery is to exclude the presence of malignancy. In one series from Memorial Sloan-Kettering Cancer Center, suspicion or inability to exclude malignancy was the indication in

36% of the patients subjected to surgery (93). The surgical mortality in experienced centers is zero or nearly zero (93,97,144,146,147,148). Nevertheless, even in specialized centers the frequency of postoperative complications may be as high as 25% (93). Enucleation is the preferred approach by many groups because it allows resection of large hemangiomas with less blood loss and preservation of more hepatic parenchyma compared to resection. Enucleation is performed using the interface between the hemangioma and the surrounding normal liver tissue

(38,92,93,149,150,151,152). Laparoscopy is currently used as the procedure of choice in many centers when the diagnosis is well established (93,146).

Although surgical resection remains the main definitive treatment for symptomatic hemangioma, other less effective options are occasionally used. Transarterial embolization has been used for symptomatic lesions (89,153), acute bleeding (154), and consumption coagulopathy (155). However, there is little data on its long-term efficacy, and the procedure may have to be repeated or may lead to liver abscesses (156). Transarterial embolization was used preoperatively to decrease blood supply to the hemangioma and improve the safety of the surgery (155). Hepatic artery ligation (93), radiation therapy (88,157), radiofrequency ablation (158), and liver transplantation (159,160,161) were used on rare occasions for unresectable giant hemangiomas. Recurrence has been described after surgical, intra-arterial, and radiation therapy (88,156).

Hepatocellular Adenoma

Epidemiology and Risk Factors

Hepatocellular adenoma, also termed *liver cell adenoma* or *hepatic adenoma*, is a benign tumor of epithelial origin occurring primarily in women of childbearing age. It is considerably less common than hemangioma or FNH, occurring in less than 0.004% of the population at risk. Adenomas may be solitary or multiple and may reach more than 20 cm in size. In contrast with hepatic hemangioma and FNH, in which the association with OCPs is variable and controversial, hepatocellular adenoma has very strong association with OCPs and other estrogens. Hepatocellular adenoma was rarely encountered before the introduction of OCPs in the 1960s. Shortly after their introduction, the incidence of hepatocellular adenoma has increased significantly (28,162,163). The annual incidence in long-term users of OCPs has increased to 3 to 4 per 100,000 compared to 1 to 1.3 per million in nonusers (164). The use of OCPs with high hormonal potency, use for more than 5 years, and age over 30 years may further increase the risk (164,165). However, in 10% of patients diagnosed with hepatocellular adenoma the exposure to OCPs may be as short as 6 to 12 months (166). Androgen steroids, anabolic steroid, and diabetes mellitus have also been associated with increased incidence of hepatocellular adenoma (167,168,169).

Hepatocellular adenomas (typically multiple lesions) are commonly encountered in association with type I and III glycogen storage diseases (170,171,172). The incidence is 22% to 75% in type I and 25% in type III (171,173,174). In sharp contrast to hepatocellular adenomas in general, which show a strong female preponderance, those associated with glycogen storage disease show a male-to-female ratio of 2:1 (170). Furthermore, adenomas associated with glycogen storage diseases develop before the age of 20.

A condition in which more than ten lesions are present has been termed *liver adenomatosis* (175,176). This condition has been reported in men and women in the absence of OCP use or glycogen storage disease (175,176,177,178).

Pathogenesis

The pathogenesis of hepatocellular adenoma is still unclear. The strong association with OCPs and pregnancy suggests a possible trophic effect of estrogen on this tumor (179). Adenomas tend to be larger and are more likely to bleed in patients on OCPs than in those with no history of OCP intake (28,163). Furthermore, regression of adenomas after discontinuation of OCPs is well documented (179,180). It has been assumed that there may be hormone receptors within the cytoplasm or nucleus

of adenoma cells that mediate tumor growth in response to stimulation. However, there has been lack of consensus about the presence of such receptors, and a study using specific monoclonal antibodies showed no evidence of their existence in adenoma cells (181). Furthermore, single or multiple adenomas may occur in men and children without known predisposing factors.

The association of adenomatosis with glycogen storage disease and diabetes mellitus has also been unclear and various theories have been suggested as possible explanations (169,170). Recently, a germline mutation of the hepatocyte nuclear factor (HNF)-1α has been described in two families that exhibited both liver adenomatosis and diabetes mellitus (169). The analysis of tumor cells showed biallelic inactivation of HNF-1α.

Pathology

Hepatocellular adenoma is usually a large, well-circumscribed yellow to light-brown tumor. It arises in an otherwise normal liver and a complete or partial capsule is frequently present, although it may occasionally be absent. It is typically solitary but may be multiple, and it ranges in size from 1 to 30 cm, most being 8 to 15 cm

in diameter (19,28,165,182,183,184). Adenomas tend to be larger in women taking OCPs. They occur more commonly in the right lobe and are usually subcapsular, projecting slightly from the surface. Occasionally, they may be pedunculated. The cut surface may show ill-defined lobulation but is never nodular or fibrotic. Foci of central hemorrhage or necrosis are frequently observed.

Microscopically, this tumor is composed of cells closely resembling normal hepatocytes. Frequently, these cells may look slightly atypical and may be either larger or smaller than the surrounding normal hepatocytes; however, they may also appear indistinguishable from normal liver cells. Adenoma cells may also contain more glycogen than the surrounding liver and hence appear relatively pale. Occasionally, they may have a relatively increased nuclear/cytoplasmic ratio, resembling well-differentiated HCC. They are arranged in plates separated by sinusoids, which may be focally dilated. The plates may be thicker than those in normal liver tissues (more than two cells). Bile-containing acinar structures may be seen, and as many as 10% may show giant cell transformation (183,184). A variety of cytoplasmic changes and inclusions can be seen, including Mallory hyaline, lipofuscin, and giant mitochondria. Typically, there are few or no portal tracts or central veins within the adenoma (19,28,183,184). Vascular elements, particularly thick-walled arteries and arterioles, are seen at the periphery of the tumor. Peliosis hepatis may also be seen.

Absence of Kupffer cells was initially reported in hepatocellular adenoma; however, studies using immunoperoxidase staining for lysozyme demonstrated the presence of Kupffer cells in variable numbers within the adenoma (183,184,185). Nevertheless, hepatocellular adenomas usually do not demonstrate the uptake of ^{99m}Tc sulfur colloid, which is typically taken up by Kupffer cells. Possible explanations are decreased numbers of Kupffer cells in some adenomas, decreased function of Kupffer cells (183,184,185), or altered blood flow in some lesions (186).

Adenomas are perfused predominantly by peripheral arterial feeders. The arterial perfusion and their hypervascular nature, with dilated sinusoids and poor connective tissue support, may explain their tendency to bleed. A percutaneous needle liver biopsy carries a high risk of bleeding and may not be diagnostic because of a lack of pathognomonic features (187).

Clinical Manifestations and Complications

Hepatocellular adenoma may present in different ways, and there are conflicting numbers in the literature with regard to the frequency of each type of presentation. In most published studies and case series, most of the diagnosed patients presented with abdominal symptoms (28,182,188); however, this may be due to a selection bias. The typical patient is a woman aged between 20 and 50 years who has a long history of OCP use, often more than 5 years (31). Typically, the symptomatic patients present with abdominal symptoms, most commonly pain or discomfort in the epigastrium or right upper quadrant. This may be accompanied by anorexia, nausea, and vomiting. The pain may be severe or sudden in onset in the setting of an acute hemorrhage or rupture (10,28,182). In these cases, the patient may present with hypotension and shock due to rapid blood loss. Mortality may be as high as 6% (28,148,189). The general risk of bleeding has not been established accurately. It increases considerably in large adenomas, in rapidly growing ones, and in patients on OCPs or after menstruation (165,188). It has been reported to be as high as 25% to 30% in patients at high risk.

In some patients, the tumor is asymptomatic and may be detected as an incidental finding on abdominal imaging study or surgery. These tend to be the smaller adenomas and those that are not associated with OCPs. Others (as many as 25%) present with an abdominal mass or hepatomegaly.

Hepatic biochemical tests are usually normal. Occasionally, serum levels of alkaline phosphatase and γ-glutamyl transpeptidase may be elevated. This occurs more commonly in patients with bleeding or rupture (6,190).

Malignant transformation of hepatocellular adenoma is uncommon but well documented (177,189,191,192). It may occur despite the discontinuation of OCPs (193,194). An enlarging lesion, local and systemic symptoms, progressive abnormality in hepatic biochemical tests, and rising blood levels of α-fetoprotein may all be signs of transformation to HCC.

Patients with adenomatosis (more than ten lesions) usually present with abdominal pain and hepatomegaly (175). They commonly have elevated blood levels of alkaline phosphatase and γ-glutamyl transpeptidase, and their tumors are more likely to bleed, rupture and undergo malignant transformation (163,175).

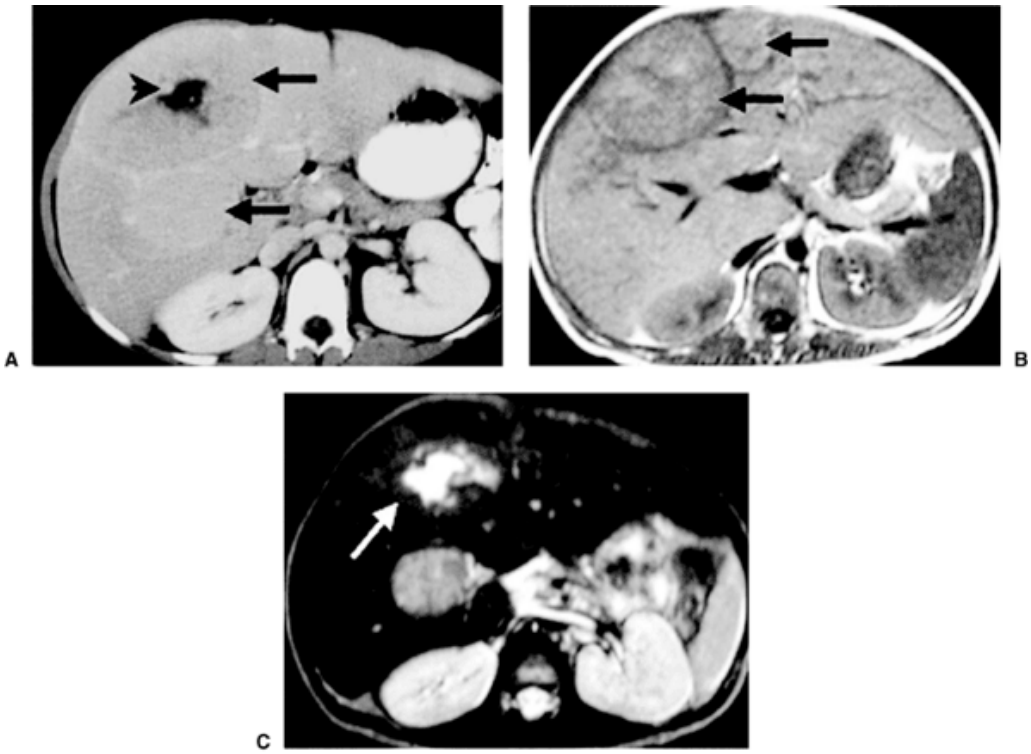
Imaging Studies

Ultrasonography shows a well-demarcated mass with smooth borders and variable echogenicity (40,110). Doppler may show venous signals within the lesion that correspond to intratumoral veins (195). However, despite the development of color Doppler, power Doppler imaging, and intravenous–ultrasound contrast agents, the findings on ultrasonography and Doppler are nonspecific and further studies are required.

CT scan does not provide a diagnostic picture either, although findings on multiphasic contrast-enhanced CT scan can help in suggesting the diagnosis (48,196). On precontrast images, the adenoma appears as a hypo- or isodense mass, which may show high-density areas, consistent with intratumoral hemorrhage (51,196,197). After contrast injection, the mass shows irregular enhancement with areas of normal, increased, and decreased density. This characteristic finding occurs in 75% of patients with hepatocellular adenoma (76) (Fig. 42.8A). The center may be hyperdense on contrast injection in the setting of an acute hemorrhage. In the presence of an old hemorrhage, the center usually remains hypodense after contrast injection, making the differential diagnosis from a central scar difficult. On the portal–venous phase, the lesion will usually show a uniform enhancement, which is

suggestive of the diagnosis (197).

MRI shows a well-demarcated lesion that on T1-weighted images may show a mildly hypointense to mildly hyperintense signal (Fig. 42.8B) (57,198,199). T2-weighted images show isointense to slightly hyperintense signal that may be heterogenous because of areas of bleeding. A peripheral rim is seen in 30% of patients (76). Central necrosis typically appears as low signal intensity on T2-weighted images, whereas a recent hemorrhage may show increased signal on T1 images (Fig. 42.8C). Gadolinium enhancement shows an arterial phase blush followed by a rapid fading in the venous and delayed phases to hypointensity or isointensity, with persistence of an enhancing rim related to the capsule. These features may be indistinguishable from those of HCC. In contrast to FNH, the signal intensity is usually not increased in the center of the lesion on gadolinium-enhanced images. An enhancing scar may be observed in a minority of patients with adenoma, but this scar does not produce a high signal on T2-weighted images as in FNH.



▪ **Figure 42.8** Hepatocellular adenoma. **A:** Abdominal computed tomography (CT) scan shows two well-circumscribed mass lesions in the right-hepatic lobe *(arrows)*. The anterior lesion shows central necrosis *(arrowhead)*. **B:** Abdominal magnetic resonance imaging (MRI) (T1-weighted image) of the same patient shows two lesions with slightly decreased intensity and well-defined low-intensity capsule *(arrows)*, which are suggestive of hepatocellular adenoma. **C:** T2-weighted image shows a central high signal intensity *(arrow)*, which is consistent with central hemorrhage.

Hepatocyte-selective contrast agents such as Gd-BOPTA have been shown to improve the differentiation between FNH and adenoma with a sensitivity of 80% and specificity of more than 96% (68,69). This

contrast agent is selectively taken up by functioning hepatocytes and excreted into

the bile. It results in prolonged enhancement of the liver parenchyma. In one recent study, 1 to 3 hours after contrast injection, 97% of FNH lesions appeared hyper- or isointense, whereas 100% of hepatocellular adenomas appeared hypointense (68).

^{99m}*Tc sulfur colloid scintigraphy* may be helpful in the diagnosis of hepatocellular adenoma because it typically shows no uptake of the colloid. This has been attributed to decreased activity of Kupffer cells within the adenoma (183,184,185) and may assist in distinguishing adenoma from FNH, which typically shows normal or increased colloid uptake.

Catheter angiography has been replaced in many centers by advanced imaging techniques such as CTA and MRA. It is used occasionally in problematic cases to rule out HCC, or as part of preoperative evaluation. In hepatocellular adenoma catheter angiography, CTA and MRA typically demonstrate enlargement of the hepatic artery and displacement of the hepatic vessels; however, these findings also appear in up to 40% of patients with FNH. In contrast to FNH the blood flow in adenoma is from the periphery to the center of the lesion, creating a homogeneous blush during the venous phase (189). Similar to FNH, adenoma may show a central hypovascular area because of hemorrhage or central necrosis. Vascular leakage, arteriovenous shunting, and portal-venous invasion suggest a malignant lesion (189).

Diagnosis

The diagnosis of hepatocellular adenoma is usually made in the setting of women aged 20 to 50 years, with a long history of OCP use, and symptomatic focal hepatic lesions.

Although many radiologic features of hepatocellular adenoma mimic HCC, the latter is usually associated with a background of chronic liver disease, usually with evidence of cirrhosis. In patients with risk factors for HCC such as chronic hepatitis B or cirrhosis, any suspicious focal lesion with radiologic features consistent with both HCC and adenoma should be regarded as HCC until proved otherwise. High or increasing serum levels of α-fetoprotein, significant increase in size, abnormal hepatic biochemical test results, and involvement of the portal vein may direct toward the diagnosis of HCC, whereas a history of long-term use of OCPs points toward hepatocellular adenoma. Despite the advanced imaging modalities, the diagnosis remains uncertain in many patients and histologic examination may be necessary. In hepatocellular adenoma, a percutaneous needle liver biopsy carries a high risk of bleeding and should be avoided. A laparoscopic approach may be necessary to improve visualization and hemostasis.

Hepatic adenomatosis (more than ten lesions) should be considered a diagnosis of exclusion because metastatic disease and multifocal HCC are more common causes of multiple solid liver masses. Histologic confirmation is required to establish the diagnosis (200).

Management

Generally, surgical treatment is recommended whenever possible to avoid the risks of rupture, hemorrhage, and malignant transformation (31,152,189,201). Some authors have suggested that adenomas smaller than 5 cm in size are less likely to rupture and bleed and, therefore, should not be resected, and should be followed by periodical imaging studies (202,203). Others have recommended a period of observation after OCPs have been discontinued to assess whether the tumor size decreases (126,202). Hepatocellular adenomas have been shown to decrease in size and even disappear after withdrawal of OCPs (28,173,180,193,204,205,206). However, there are rare reports of cancer occurring even in adenomas that have decreased in size or disappeared after discontinuation of OCPs (193,194). We

therefore recommend, like others, surgical resection for all hepatocellular adenomas if technically possible (192). Nevertheless, the risk–benefit ratio should be assessed for every case and the final decision should be made on an individual basis.

Surgical approaches for hepatocellular adenoma have included enucleation, resection, and liver transplantation (21,38,189,201). Enucleation is associated with less blood loss and preservation of normal liver tissue (207). Both resection and enucleation are performed laparoscopically in many centers with excellent results, when technically feasible (82,201). The mortality of an elective surgery is 1%, but it increases to 5% to 8% for emergency resection (82,207,208,209). Intraperitoneal bleeding from hepatocellular adenoma has been associated with 21% mortality (164). Arterial embolization has been used to control bleeding (202), reduce the tumor size preoperatively, or relieve symptoms in patients with inoperable tumors (189,210).

In rare patients with multiple adenomas, orthotopic liver transplantation (OLT) may be the only way to remove all the lesions (171,177). In type I glycogen storage disease OLT also corrects the metabolic defect (211).

If an adenoma is not resected, the patients should be followed up periodically by imaging studies and by determining the blood levels of α-fetoprotein, although there is scarce evidence to support the efficacy of this approach (171). Local or systemic symptoms, any increase in size, and any abnormality in hepatic

biochemical tests or α-fetoprotein levels should raise the suspicion of malignant transformation and should prompt a surgical intervention to remove the lesion. In addition, pregnancy should be avoided to prevent the risk of rapid tumor growth, rupture, and bleeding (194). Women who have had hepatocellular adenoma, whether resected or not, should avoid the use of OCPs permanently.

In patients with glycogen storage disease, pregnancy is probably safe. In the few reports of patients with glycogen storage disease and hepatocellular adenomas who became pregnant, there was no change in the size or number of the adenomas (212). This observation supports the notion that the pathogenesis of hepatocellular adenoma in patients with glycogen storage disease may be different from that of adenomas occurring in healthy individuals.

Macroregenerative Nodules

There is ongoing discussion about the characterization and classification of hepatocellular nodules found in the cirrhotic liver. Improvement of imaging techniques has led to frequent detection of focal lesions in cirrhotic livers, many of which are benign nodules with various degrees of atypia. They may occur in cirrhotic livers of any cause. Several groups have suggested nomenclatures and diagnostic criteria to describe these lesions; however, none of these are used consistently (213,214). Terms such as *regenerative nodules*, *macroregenerative nodules* (*MRNs*), *adenomatous hyperplastic nodules* (215,216), *dysplastic nodules* (213), *atypical adenomatous hyperplastic nodules* (217), and *borderline nodules* (214) have been suggested by different authors to describe what is believed to be different stages of the same lesion.

An MRN is typically larger than 0.8 cm. It develops because of the regeneration of an islet of viable cells within an area of destroyed liver tissue, hence its nodular appearance. MRNs may be seen in less than 2% of cases of acute massive necrosis and in 14% to 21% of patients with cirrhosis (218). The expanding nodule typically compresses the surrounding liver tissue. It may be solitary but many livers contain multiple MRNs. It is separated from the rest of the liver parenchyma by fibrous connective tissue. Needle biopsy specimens from these lesions are commonly

classified as benign, but in some cases they may demonstrate varying degrees of atypia and occasionally foci of overt HCC. The characteristic histologic findings of MRN include normal thin plates of hepatocytes (two cells thick) and lack of cytologic atypia (normal nuclear/cytoplasmic ratio). The nodule may include multiple portal areas. Larger hepatocytes may be seen and are considered an acceptable finding as long as the normal nuclear/cytoplasmic ratio is maintained.

MRNs have been classified according to degree of atypia to type I and type II (219). Type I MRN shows no cell atypia and is probably an ordinary adenomatous hyperplasia, whereas type II MRN represents atypical adenomatous hyperplasia, which is probably a true precursor of HCC (219). The clonality of MRNs suggests that at least some of them are neoplastic and probably premalignant (217). The incidence of progression to malignancy is still unclear (220,221,222,223); however, some authors recommend treating atypical (type II) MRN in a patient with cirrhosis as a malignant lesion (221). In a study of 307 patients with cirrhosis, multivariate analysis showed dysplasia to be the most important independent risk factor for the development of HCC (224).

Imaging studies may be suggestive of MRN, although they may not be sufficient to establish the diagnosis or distinguish MRN from HCC. On T1-weighted MRI, MRNs are typically isointense to slightly hyperintense compared to the surrounding hepatic parenchyma. On T2-weighted images the lesions are hypointense. In contrast, HCC has variable diminished signal intensity on T1, becomes hyperintense on T2, and enhances during the arterial phase of gadolinium injection. HCC also has a characteristic capsule of low signal intensity (57,124), which is typically absent in MRNs. MRA usually shows sparse arterial supply in MRN. Uniform arterial opacification is seen without neovascularity. The transition of MRN to dysplastic nodule and subsequently to HCC is associated with vascular proliferation within the nodule and preferential blood supply from branches of the hepatic artery (57,225).

Benign Focal Lesions of Bile Duct Origin

Two main types of intrahepatic benign bile duct proliferation have been characterized: Bile duct adenomas (184,226) and bile duct hamartomas (also called *von Meyenburg complexes*).

Intrahepatic Bile Duct Adenoma

Intrahepatic bile duct adenoma is a rare non-neoplastic focal lesion that is usually discovered incidentally. Most cases are reported in men older than 40 years. Typically, it is a solitary subcapsular lesion measuring 0.5 to 2 cm in size (227). Microscopically, it consists of numerous, uniform, normally appearing bile duct–like structures that are surrounded by a small amount of fibrous stroma (184). This lesion is occasionally confused with cholangiocarcinoma or metastatic adenocarcinomas on frozen sections. The distinction should be made by the absence of nuclear hyperchromasia, mitotic activity, and vascular invasion (13,184). It is believed that bile duct adenoma is a reactive process to a focal injury rather than a true neoplasm or a developmental anomaly (227).

Biliary Hamartoma (Von Meyenburg Complex)

Biliary hamartoma is an anomalous proliferation of dilated bile ducts embedded in connective tissue. It is often visible grossly as a tiny (up to 0.5 cm) white liver nodule and may be single or multiple (228). Biliary hamartoma is most often seen in otherwise normal liver tissue, but it may be associated with congenital hepatic fibrosis and Caroli's disease. There is also a strong association with adult polycystic

disease. It is usually detected as an incidental finding at surgery, autopsy, and rarely percutaneous liver biopsy performed for other reasons. Its pathogenesis likely involves developmental arrest of the primitive ductal plate. The ectatic ducts may contain bile or even small calculi. Von Meyenburg complex was found in 5.6% of adults in 2,843 autopsies. Of the patients with von Meyenburg complex, 11% had adult polycystic kidney disease, whereas 97% of the patients with adult polycystic kidney disease had the von Meyenburg complex (229). There is a rare association with cholangiocarcinoma (230).

Other Benign Mesenchymal Tumors

Infantile Hemangioendothelioma

Infantile hemangioendothelioma is a vascular tumor derived from endothelial cells, which is the most common benign hepatic tumor in children. It accounts for approximately 12% of all childhood hepatic tumors and for more than 50% of benign tumors of the liver diagnosed in infancy and childhood (231). It can be solitary (55% of the patients) or multiple (45% of the patients) and may vary in size from a few millimeters to more than 20 cm (183,184). More than 90% are diagnosed before the age of 6 years. The typical presentation is of hepatomegaly, hemangiomas of the skin, and heart failure resulting from massive arteriovenous shunting (232). In addition to heart failure, this tumor may cause consumption coagulopathy (Kasabach-Merritt syndrome) and obstructive jaundice (102,233).

Although well circumscribed, this tumor is not encapsulated and often has scattered calcifications. Microscopically, this tumor consists of multiple small vessels lined by plump endothelial cells and surrounded by fibrous stroma. Two types of hepatic hemangioendotheliomas exist. The more common, type I, has a prominent endothelium closely related to the portal tract and tends to displace, rather than infiltrate, the liver parenchyma. It consists of small vascular channels lined by flattened or rounded endothelial cells with rare mitotic figures. Extramedullary hematopoiesis may occasionally be seen. Type II lesions are composed of tortuous vascular channels, with endothelial cells proliferating into the adjacent hepatic tissue. They exhibit larger, irregular channels lined by pleomorphic endothelial cells. Differentiation of type II hemangioendothelioma and the rare malignant angiosarcoma may be difficult. In many cases, infantile hemangioendothelioma is associated with extrahepatic hemangiomas that may be found in the skin, lung, lymph nodes, pancreas, retroperitoneum, and bone. The most frequently involved site is the skin, where single or multiple lesions may be present. Other associated abnormalities include atrial septal defect, patent ductus arteriosus, myelomeningocele, renal agenesis, and absent common bile duct (233). Rarely, infantile hemangioendothelioma may undergo transformation to angiosarcoma (234).

The diagnosis is suggested when an infant presents with an enlarged liver, congestive heart failure, and cutaneous hemangiomas (235). The bilirubin level is elevated in one third of patients. Biliary obstruction is rarely present (233,236).

Ultrasonography usually shows hepatomegaly and solitary or multiple hepatic lesions, which may vary from anechoic to hyperechoic. The unenhanced CT scan demonstrates the lesion as a well-defined hypoattenuating mass, occasionally with calcifications. After contrast injection the lesion may show enhancement resembling hemangioma and may become isodense on delayed images. Angiography shows dilated, irregular vascular lakes that commonly persist beyond the venous phase. ^{99m}Tc sulfur colloid scintigraphy shows the lesion as a cold spot because of lack of Kupffer cells within the tumor.

The prognosis of this lesion is dependent on its size and its effect on the heart

function. Spontaneous regression is frequent but death may occur within the first 6 months of life because of cardiac failure or replacement of the normal hepatic parenchyma (102). The prognosis is usually good if heart failure is managed successfully. In one large series, the 6-month survival was 70% (237).

The treatment is dictated by tumor-related symptoms produced by tumor size. Management of congestive heart failure may be sufficient in some cases. If symptoms are not relieved, treatment should be aimed at decreasing the tumor size. Several studies have confirmed the success of steroid therapy (238). However, failure of some tumors to respond to steroids has led to the use of other drugs such as cyclophosphamide (239) and interferon α-2a (240). Other treatments include hepatic artery ligation (241), transcatheter endovascular embolization (242), and radiation therapy (243). Liver transplant is increasingly recognized as a viable treatment modality for infantile hemangioendothelioma when other treatments fail (244).

Mesenchymal Hamartoma

Mesenchymal hamartoma is a rare slow-growing tumor of childhood that comprises approximately 5% of pediatric liver tumors. It has a male predominance and is diagnosed in most cases before the age of 3 years (245,246,247), although it has been reported in adults (248). The tumor is usually a solitary, large (average size of 16 cm), well-demarcated mass with a predilection for the right hepatic lobe. The cut surface shows multiple cysts separated by solid, pink tan areas. The solid portions have a mixture of hepatic cells and mesenchymal cells with vascular proliferation and bile duct–like structures. The cystic spaces are filled with fluid or solid gelatinous material. The islands of hepatocytes within the mesenchymal stroma may show reactive and regenerative changes (184). Most patients present with abdominal enlargement or an abdominal mass. Few have pain or respiratory distress. Ultrasonography and CT demonstrate a large tumor, with central cystic changes showing internal septations (245). Angiography reveals a hypovascular or avascular lesion. It is important to differentiate this tumor from embryonal sarcoma because both may demonstrate loose edematous myxoid stroma. In most cases of embryonal sarcoma, the cellularity of the tumor is readily evident, and the neoplastic cells show distinctive cytologic features of highly malignant cells. Resection is recommended when the tumor is large and compresses adjacent abdominal organs. It is curative in most cases. A simultaneous involvement of the liver by mesenchymal hamartoma and infantile hemangioendothelioma requiring liver transplantation has been reported (249).

Benign Lipomatous Tumors and Tumor-like Lesions

Focal Fatty Change

Focal fatty change is a localized area of steatosis that can sometimes be misinterpreted on imaging studies as a neoplastic growth. It is usually an ill-defined area that may be single or multiple. Multiple areas of fatty change may mimic metastatic disease on imaging studies (250). Focal fatty change is associated with alcoholism, obesity, malnutrition, total parenteral nutrition, corticosteroid treatment, cytotoxic chemotherapy, acquired immunodeficiency syndrome (AIDS), hypertriglyceridemia, and diabetes mellitus. Hepatic biochemical tests may be normal or mildly abnormal.

Ultrasonography shows a hyperechoic area with ill-defined borders. On CT scan, focal fatty change appears as a hypodense area, which is usually sharply demarcated without mass effect on the hepatic or portal veins (251,252,253). The demonstration

of normal-caliber vessels coursing through the hypodense lesion on CT scan is characteristic (254) and can help in distinguishing this lesion from primary or metastatic malignancy. MRI shows increased intensity on T1-weighted images. This finding is characteristic for focal fatty change appearing in 100% of the cases compared with less than 4% of other benign tumors. The differential diagnosis of a hyperintense focal hepatic lesion on T1-weighted images includes hemorrhage, malignant melanoma, and iron or copper overload (255,256). Fat-suppressed T1-weighted images may be helpful in identifying fatty infiltration because the hyperdense area typically becomes hypodense in a fat-containing lesion (255). ^{99m}Tc sulfur colloid scintigraphy shows no focal lesion; however, areas of steatosis show retention of xenon-133 (257,258). Biopsy is diagnostic but not always required. Microscopically, the lesion shows macrovesicular fatty change within the lesion, which is indistinguishable from other forms of steatosis.

The treatment of focal fatty change is directed against the underlying disease. Resolution has been described after weight loss and abstinence from alcohol (259).

Angiomyolipoma

Angiomyolipoma is a rare benign lipomatous tumor resembling the more common renal angiomyolipoma. It is usually solitary, ranging in size from 0.3 to more than 20 cm. It is typically asymptomatic and is detected as an incidental finding between the second and eighth decades, predominantly in women (260,261,262). It is composed of variable proportions of adipose tissue and smooth muscle with thick-walled blood vessels. Adipose tissue varies from as little as 55% to more than 90% (184). Tumors with extensive extramedullary hematopoiesis have been termed *myelolipoma* or a*ngiomyelolipoma* (263). Flow cytometry shows a deoxyribonucleic acid (DNA) diploid pattern, consistent with a benign lesion (264).

Angiomyelolipoma is a homogenous well-circumscribed tumor that is highly echogenic on ultrasonography. On enhanced CT scan, the density measurements are characteristic of fat (-2 to -115 Hounsfield units) (265); MRI is also diagnostic. The prognosis is usually excellent. Budd-Chiari syndrome related to compression of the hepatic veins by angiomyelolipoma has been described (266,267). Malignant transformation has not been reported. For symptomatic lesions or lesions in which the diagnosis cannot be confirmed, resection is the treatment of choice (260).

Pseudolipoma

Pseudolipoma is a small encapsulated lesion located on the capsular surface of the liver or immediately under the capsule. It consists of mature lipocytes and is presumed to originate from adherent epiploic appendices that became detached. Fat necrosis and calcifications may occur. The importance of this rare and asymptomatic lesion lies in its differential diagnosis from primary or metastatic tumors. There are no clear predisposing factors (184,268).

Inflammatory Pseudotumor

Inflammatory pseudotumor of the liver is a rare benign disease presenting as a localized parenchymal mass. It is considered by some to be secondary to an infectious process (269,270), although the exact pathogenesis remains unclear, and few microscopic studies of hepatic tissue have disclosed pathogenic microorganisms (271). An association with extrahepatic infectious conditions (270) and with Crohn's disease (272,273) has been reported in a few patients. The lesion may be solitary or multiple and its size ranges from 1 to 25 cm. It is usually well circumscribed and may be encapsulated. It affects patients at any age and has a predilection for men

with a male-to-female ratio of 3 to 8:1.

The patients usually present with fever, abdominal pain, and malaise. Jaundice and weight loss may occur. Elevated sedimentation rate, leukocytosis, and mildly abnormal hepatic biochemical tests are common. Ultrasonography reveals a heterogeneous lesion that may show a mosaic pattern. CT scan shows an irregular, clearly demarcated heterogeneous mass that is typically hypodense compared to surrounding liver parenchyma.

The differential diagnosis includes pyogenic hepatic abscess, as well as malignant and metastatic tumors. Needle liver biopsy is usually needed to establish the diagnosis.

Histologically, the mass is composed of fibrous tissue and myofibroblasts and infiltrated by dense mixed inflammatory infiltrate with numerous plasma cells. Vascular invasion can sometimes be seen, and phlebitis may be present (184).

The prognosis of hepatic inflammatory pseudotumor is usually favorable. The lesion may regress and disappear spontaneously within a few weeks to months (270,274,275,276,277). In a few cases antibiotic treatment was used and was followed by complete resolution (270,271). Response to steroids has also been reported. In some cases the lesion was treated successfully by local resection (278), but surgical removal is unnecessary in most patients. When the diagnosis is unequivocal, observation is the treatment of choice (270,274,275,276,277).

Clinical Approach to a Focal Solid Lesion of the Liver

Focal solid hepatic lesions often represent a diagnostic challenge and frequently mandate an extensive evaluation and a multidisciplinary approach. Although there is a wide spectrum of conditions that needs to be considered when a focal lesion is detected in the liver, most lesions fall into a relatively small group of entities (Table 42.1). The clinician must decide at an early stage whether the lesion is malignant or benign, whether a biopsy is indicated, and whether surgical intervention is required.

A detailed history should be obtained and should include questions about the history of cancer and previous use of OCPs, androgens, and anabolic steroids. The specific approach may vary according to the type of presentation, demographics of the patient, and medical history. The diagnostic approach in a young, asymptomatic previously healthy woman should focus on the differential diagnosis of hemangioma, FNH, and adenoma. A history of prolonged treatment with OCPs will point toward a diagnosis of hepatocellular adenoma. In contrast, when the patient has a history of cancer, a focal hepatic lesion will often be a metastasis. In the presence of cirrhosis or chronic hepatitis B virus (HBV) infection, a focal solid lesion should be regarded as HCC until proved otherwise. A history of primary sclerosing cholangitis should point toward intrahepatic cholangiocarcinoma.

Presenting symptoms such as abdominal pain, weight loss, and malaise should raise the suspicion of malignancy. It should be remembered that most patients with benign hepatic lesions are asymptomatic, and the relationship between the symptoms and the focal lesion should be corroborated.

A thorough physical examination is important to assess whether an underlying liver disease or malignancy is present. Physical findings such as ascites, firm nodular liver, or splenomegaly should suggest the presence of a chronic liver disease and should point toward HCC. Hepatic biochemical tests are typically normal in patients with benign hepatic lesions, and a significant abnormality should raise the suspicion of a neoplastic lesion, a complication such as hemorrhage (usually in an adenoma), or an underlying liver disease. Tumor marker levels are also normal and are unhelpful in making the distinction between benign tumors. Nevertheless, any

abnormality in tumor marker should prompt an evaluation for malignancy.

If the patient has no history of cancer, no underlying liver diseases, and no abnormality in tumor marker levels or serologic tests for viral hepatitis, a solid lesion of the liver is most often benign (279,280).

Imaging studies are commonly helpful in distinguishing between focal hepatic lesions, but the differential diagnosis may be challenging because many of the lesions have overlapping radiographic features (48,57,281).

Although ultrasonography is an excellent modality for the initial detection of lesions and for determining a solid versus cystic lesion, the sonographic appearance of a solid liver mass is often nonspecific and many patients will require additional hepatic imaging. CT is a reliable and easily reproducible method for the evaluation of focal hepatic lesions. It allows the characterization of lesions as small as 1.5 cm in size and may also help in the evaluation of the entire abdomen for signs of cirrhosis, portal hypertension, or malignancy (48,282). CTA and CT arterial portography show improved sensitivity and may detect smaller lesions; however, MRI has the advantages of specificity and the ability to distinguish between different lesions (283). The use of specific MRI contrast media, such as SPIO and Gd-BOPTA has been shown to improve specificity and facilitate the differentiation between FNH, adenoma, and other lesions.

In a patient with cirrhosis or chronic HBV infection and a solid lesion, arterial enhancement on dynamic CT scan strongly supports the diagnosis of HCC (173). When a hemangioma is suspected, triphasic dynamic CT with late images should be performed. Dynamic CT scan may conclusively establish the diagnosis of a hemangioma and, occasionally, FNH without any further workup (284). Tagged RBC scan (^{99m}Tc-RBC pool scintigraphy) may be helpful in questionable hemangiomas, although gadolinium-enhanced MRI is superior to tagged RBC scan in most cases. ^{99m}Tc-RBC scan with SPECT is highly sensitive in hemangiomas larger than 2 cm in size and may be used to confirm the diagnosis. ^{99m}Tc sulfur colloid scintigraphy is usually not helpful for the differential diagnosis of focal hepatic lesions, but it may assist in distinguishing between FNH and adenoma. If no focal defect is seen or if the uptake is increased, FNH should be suspected. In contrast, decreased uptake is more consistent with an adenoma. PET scan proved to be highly sensitive in detecting hepatic metastases and distinguishing them from other focal lesions (285). At this point in time, PET scan has not been shown to have an advantage over current imaging modalities for the diagnosis of specific benign tumors. The finding of a central scar in a solid lesion, although suggestive of an FNH, may be misleading and should not be confused with a central hemorrhage or necrosis. Calcifications within a lesion suggest fibrolamellar carcinoma, whereas hemorrhage within a tumor is more suggestive of an adenoma.

In cases of uncertainty or when malignancy is suspected, a liver biopsy should be performed. A percutaneous liver biopsy is generally safe; however, it may be hazardous in adenomas and hemangiomas and should be avoided when these tumors are suspected, or when imaging studies indicate hypervascularity. In these cases, laparoscopic liver biopsy may be safer and may have the advantage of hemostasis under direct vision. Laparoscopy also offers a better evaluation of the extent of the lesion through direct vision and laparoscopic ultrasonography (286).

In a minority of patients, an accurate diagnosis cannot be established despite histopathologic examination. In these cases, resection may be indicated (208,287). Similarly, persistent symptoms, increasing levels of tumor markers, or evidence of tumor growth are indications for a surgical resection, regardless of the diagnosis. Benign hepatic lesions accounted for 5% of hepatic resections in a recent database

review in the United States (288). Resection of benign lesions is performed laparoscopically in many centers with excellent results. A recent European study reported 87 laparoscopic resections of benign tumors with zero mortality and a postoperative complication rate of 5% (82).

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Chapter 43
Nodular and Cystic Lesions

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Key Concepts

- Dysplastic nodules are defined as a nodular region of hepatocytes at least 1 mm in diameter with dysplasia but without definite criteria of malignancy. When such nodules are larger than 10 mm, the term *macroregenerative nodule* (*MRN*) is applied.
- Nodular regenerative hyperplasia (NRH) is defined by hepatocellular nodules that are distributed throughout the liver in the absence of fibrous septae between the nodules. This condition is often associated with systemic diseases and is complicated by portal hypertension.
- Focal nodular hyperplasia (FNH) is a benign tumor-like lesion of the liver considered to be hyperplastic rather than neoplastic in origin. This lesion is related to an abnormal blood supply and is rarely of clinical significance.
- Hepatocellular adenoma is a benign neoplasm of the liver, often related to the use of oral contraceptives. This neoplasm can be complicated by rupture, hemorrhage, and, rarely, development of malignancy.
- Polycystic liver disease (PCLD) is an inherited disease in which multiple cysts develop within the parenchyma of the liver and produce disease from direct mechanical effects of the cysts.
- PCLD is often associated with other related diseases, such as autosomal dominant polycystic kidney disease (ADPKD), whereas the rarer congenital hepatic fibrosis (CHF) and choledochal cysts may occur in association with autosomal recessive polycystic kidney disease (ARPKD).
- PCLD is usually asymptomatic but can be complicated by the development of abdominal pain, portal hypertension, hepatic venous outflow obstruction, obstructive jaundice, and cyst infection.
- Cystadenoma is a benign tumor of the liver that is hypothesized to arise from congenital defects of the bile ducts or gallbladder. This condition carries a high risk of development of malignancy.
- Echinococcal cysts are related to infection with the parasites *Echinococcus granulosus* and *Echinococcus multilocularis* and may affect many organs, including the liver.

Nodular Diseases of the Liver

The classification and terminology of nodular diseases of the liver is somewhat confusing because different terms have been applied to the same type of lesion. In an attempt to standardize the terminology, the International Working Party proposed a new nomenclature, which has been widely adopted by pathologists (1). However, this terminology does not always lend itself to everyday clinical use, and the following discussion represents an attempt to combine this new standard terminology with the clinical features of hepatic nodules.

Hepatocellular Nodular Lesions with Cirrhosis

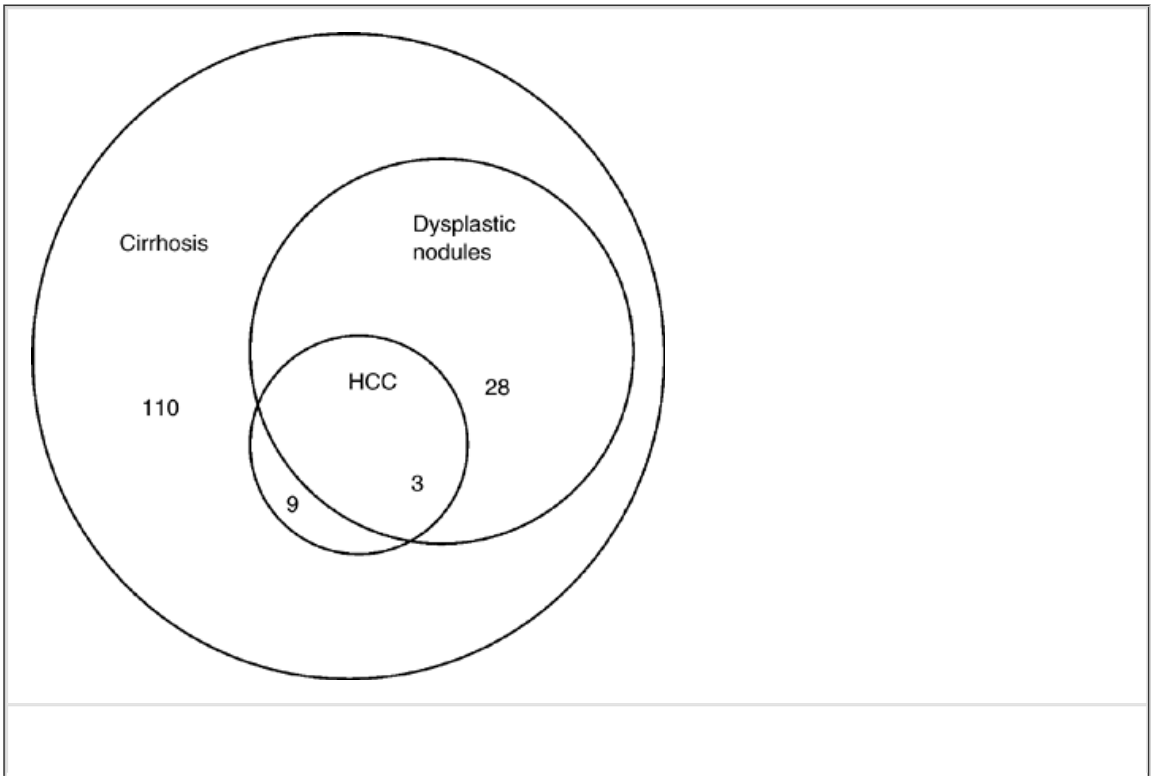
The definition of cirrhosis has three components based on gross or microscopic pathologic findings: (a) Nodules of regenerating hepatocytes (b) bands of fibrosis that surround these nodules, and (c) the process by which this occurs, which is diffuse throughout the liver. Many of the other forms of nodules described later are differentiated from cirrhosis by not meeting one of these three criteria. Cirrhosis is usually classified on the basis of morphologic findings as being *micronodular*, *macronodular*, or *mixed*. These terms refer to the sizes of the nodules of regenerating hepatocytes that are present. Therefore, in micronodular cirrhosis, the nodules are usually 2 to 3 mm in diameter. This pattern is characteristic of the cirrhosis caused by alcohol and hemochromatosis, whereas most other causes of cirrhosis result in macronodular or mixed macronodular and micronodular cirrhosis, in which the nodules typically range between 3 and 10 mm in diameter.

Dysplastic nodules

Among cirrhotic nodules, some stand out as being unusual on gross or microscopic examination. Therefore, dysplastic nodules are defined as a nodular region of hepatocytes at least 1 mm in diameter with dysplasia but without definite criteria of malignancy. For nodules larger than 10 mm, the term *macroregenerative nodule* (MRN) has been applied. Although the International Working Party abandoned it, this term may still have some value because clinicians and radiologists often encounter large regenerative nodules that seem to have a propensity to become malignant. Synonyms for this lesion include *large regenerative nodule* and *adenomatous hyperplasia*.

In two series of cases, investigators examined the incidence of MRNs in liver explants. Ferrell et al. (2) examined 110 sequentially explanted cirrhotic livers and found that 19 of them (17.3%) had nodules between 0.8 and 3.5 cm in diameter (Fig. 43.1). Ten livers had more than 1 nodule, and a total of 40 nodules were detected. Twelve of the nodules were hepatocellular carcinoma (HCC) and 28 were MRNs. Theise et al. (3) examined 44 explanted livers and identified 48 MRNs larger than 1 cm in diameter in 11 livers. Both these studies showed a close association between MRNs and HCC in the same liver.

There is debate about the origin of dysplastic nodules. It was thought that they are simply "overgrown" cirrhotic nodules. The observation that nearly all dysplastic nodules contain intact portal triads implies that these lesions are not derived from regenerating nodules but rather that they may represent the growth of nodules of transformed hepatocytes (4).



▪ **Figure 43.1** Dysplastic nodules and hepatocellular carcinoma (HCC) found in cirrhotic livers at the time of transplantation. (Adapted from Ferrell L, Wright T, Lake J, et al. Incidence and diagnostic features of macroregenerative nodules vs. small hepatocellular carcinoma in cirrhotic livers. *Hepatology* 1992;16:1372–1381, with permission.)

Dysplastic nodules rarely result in any clinical symptoms. Patients may have clinical features associated with cirrhosis, or the nodules may be detected radiographically if they are large enough, usually as an incidental finding. Nodules larger than 1 cm in diameter can be detected with either ultrasonographic examination or sensitive computed tomographic (CT) or magnetic resonance imaging (MRI) techniques. Unfortunately, large dysplastic nodules cannot be differentiated reliably from HCC by radiographic means; therefore, they present a challenge to the clinician caring for the patient. Biopsy of such nodules can be performed with CT or ultrasonographic guidance to rule out HCC, but if cancer is not present in the specimen, the possibility that the correct portion of the lesion has not been sampled cannot be excluded. Table 43.1 shows the high rate of development of malignant growth within large dysplastic nodules, ranging between 24% and 45% over a period of several years.

Hepatocellular Nodular Lesions without Cirrhosis

Nodular regenerative hyperplasia

Nodular regenerative hyperplasia (NRH) is defined by hepatocellular nodules distributed throughout the liver in the absence of fibrous septae between the nodules. Most reports have been of single cases or small series of cases. A recent autopsy study showed that NRH was

present in 2.6% of 2,500 consecutive autopsies. NRH has been reported to occur in children and even in fetal liver. Familial occurrence has been documented. NRH has been described in association with a variety of hepatic and systemic diseases (Table 43.2) but appears to be a nonspecific tissue adaptation to heterogeneous distribution of blood flow rather than a distinct disease entity. What many of these conditions appear to have in common is the disturbance of blood flow to the liver, which can involve the portal vein (through thrombosis), the hepatic artery (with arteritis), and the hepatic outflow tract (as in Budd-Chiari syndrome and congestive heart failure). Even some of the drugs associated with NRH probably act through their effect on blood vessels. For example, azathioprine may cause signs and symptoms that resemble those of veno-occlusive disease, and the use of this agent is causally linked with NRH. It seems that insufficient blood supply to portions of the liver, caused by conditions such as portal venous thrombosis, leads to atrophy of the parenchyma, with compensatory hyperplasia occurring in areas with adequate blood supply.

Table 43.1. Outcome of Radiographically Identified Dysplastic Nodules

Author	Number of nodules	Outcome of nodules		
		Disappeared (%)	Hepatocellular carcinoma (%)	Unchanged (%)
Takayama et al. (4a)	20	2 (10%)	9 (45%)	9 (45%)
Kondo et al. (4b)	17	4 (24%)	4 (24%)	9 (52%)
Borzio et al.	32	7 (22%)	8 (25%)	17 (53%)

(4c)				

At gross examination of the liver, there is diffuse fine nodularity of the liver approximately 1 to 2 mm in diameter. Microscopic examination shows that the normal architecture has been replaced with monoacinar regenerative nodules, which often contain portal tracts (4). The nodules are surrounded by compressed liver cell plates rather than fibrosis, and dilated sinusoids may be seen adjacent to nodules (Fig. 43.2). Although the nodularity may be visible with hematoxylin and eosin stain, it is best seen with a reticulin stain, which shows thick cell plates within the nodule and compressed plates surrounding the nodule.

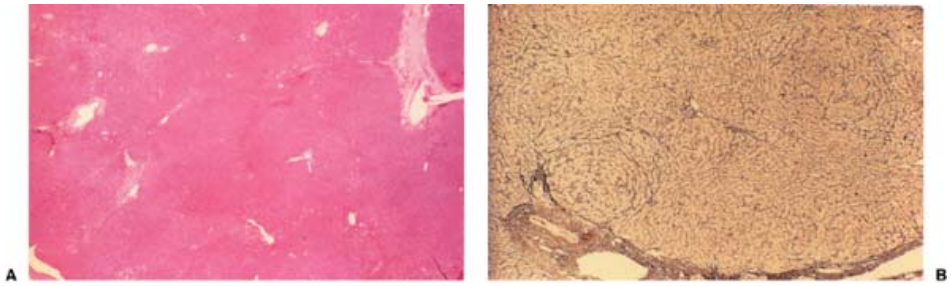
The clinical features of NRH are variable, and many patients have no symptoms. Patients range widely in age, although NRH is rare among children. Patients often have a history of another medical illness, which was presumably a predisposing factor for NRH. The main clinical consequence of NRH is portal hypertension, which manifests as splenomegaly and gastroesophageal varices. Ascites is uncommon because in most patients the hepatic synthetic function is preserved and, therefore, serum albumin levels are normal. Serum aminotransferase levels are characteristically normal, as are bilirubin values, although alkaline phosphatase levels are usually moderately elevated (5). Therapy is directed at removing the causative agent if possible and controlling portal hypertension. Patients with NRH who experience bleeding from varices usually tolerate this well because hepatic synthetic function is preserved. Surgical shunting or transjugular intrahepatic portosystemic shunt (TIPS) is rarely indicated, and the varices can often be controlled with injection,

sclerotherapy, or endoscopic banding. Patients with NRH have mistakenly undergone liver transplantation because they were thought to have cirrhosis, and they have done well (6).

Table 43.2. Conditions Associated with Nodular Regenerative Hyperplasia

Vascular disease
Budd-Chiari syndrome
Portal venous thrombosis
Drugs and toxins
Azathioprine
Thorotrast
Toxic oil syndrome
Thioguanine
Collagen vascular disease
Systemic lupus erythematosus
Scleroderma
Mixed connective tissue disease
Rheumatoid arthritis
Felty's syndrome
Polymyalgia rheumatica
Antiphospholipid antibody syndrome

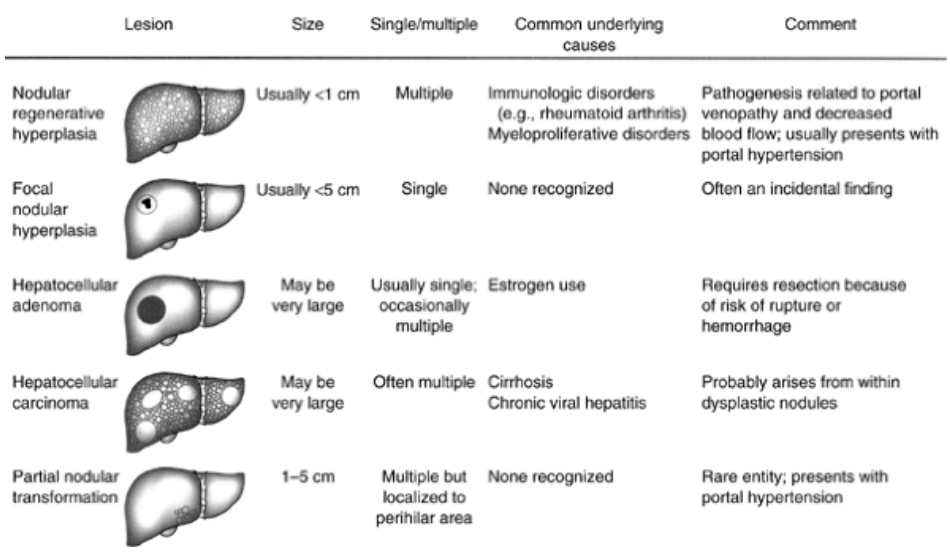
- Other liver disease
  - Primary biliary cirrhosis
  - After liver transplantation
  - Metastases from pancreatic cancer
  - Hepatocellular carcinoma
- Neoplastic conditions
  - Myeloproliferative disorders
  - After bone marrow transplantation
  - Non-Hodgkin's lymphoma
  - Castleman disease
- Immunodeficiency syndromes
  - HIV infection
  - Common variable immunodeficiency
- Miscellaneous
  - Primary pulmonary hypertension
  - Glomerulonephritis
  - Behçet's disease
  - Schnitzler syndrome
  - Diabetes mellitus
  - Congestive heart failure
- Idiopathic
  - Sporadic
  - Familial



▪ **Figure 43.2 A:** Photomicrograph of the liver shows nodules of nodular regenerative hyperplasia without surrounding fibrosis (hematoxylin and eosin). (Courtesy of Elizabeth M. Brunt, MD.) **B:** Nodular regenerative hyperplasia (reticulin stain). (Courtesy of Elizabeth M. Brunt, MD)

Unusual forms of nodular regenerative hyperplasia

Felty's syndrome is a rare complication of rheumatoid arthritis consisting of leukopenia and splenomegaly (7). Leukopenia may be associated with increased risk of bacterial infection. The splenomegaly is thought to be caused by portal hypertension. Most biopsy specimens of the liver from patients with Felty's syndrome show NRH, although a small proportion show only portal fibrosis or sinusoidal lymphocytosis. Felty's syndrome carries an increased risk of non-Hodgkin's lymphoma (8).



Lesion	Size	Single/multiple	Common underlying causes	Comment
Nodular regenerative hyperplasia	Usually <1 cm	Multiple	Immunologic disorders (e.g., rheumatoid arthritis) Myeloproliferative disorders	Pathogenesis related to portal venopathy and decreased blood flow; usually presents with portal hypertension
Focal nodular hyperplasia	Usually <5 cm	Single	None recognized	Often an incidental finding
Hepatocellular adenoma	May be very large	Usually single; occasionally multiple	Estrogen use	Requires resection because of risk of rupture or hemorrhage
Hepatocellular carcinoma	May be very large	Often multiple	Cirrhosis Chronic viral hepatitis	Probably arises from within dysplastic nodules
Partial nodular transformation	1–5 cm	Multiple but localized to perihilar area	None recognized	Rare entity; presents with portal hypertension

▪ **Figure 43.3** Nodular diseases of the liver. (Reproduced from Di Bisceglie AM, Buetow PC. Tumors of the liver. In: Maddrey WC, Feldman M, eds. *Atlas of the liver*, 2nd ed. Philadelphia, PA: Current Medicine, 2000:13.1–13.14, with permission.)

Partial nodular transformation

Partial nodular transformation (PNT) of the liver is a rare entity characterized by the formation of large hepatocellular nodules without marked fibrosis, particularly at the hepatic hilum or around large portal areas. It does not meet the criteria of cirrhosis because the condition is not diffuse and the nodules are not surrounded by fibrosis. It is similar to NRH, except that the nodules of NRH are diffuse throughout the liver and are usually much smaller (Fig. 43.3). PNT usually occurs as an isolated condition but has been reported in association

with vascular abnormalities such as persistent ductus venosus, portal venous thrombosis, and portal venous emboli of HCC (9,10,11). The pathogenesis is uncertain, but it has been suggested that PNT may represent a variant of NRH in which localized obstruction of blood flow causes formation of localized hepatic nodules (8). Because these nodules occur predominantly in or near the hepatic hilum, PNT often leads to presinusoidal portal hypertension.

PNT has been found in both adults and children. Patients usually have features of portal hypertension, such as bleeding from gastroesophageal varices and ascites. If the nodules are large enough, hepatomegaly resulting from portal hypertension may be present with splenomegaly. Serum aminotransferase activity is typically within the normal range. The diagnosis of PNT is often made only at autopsy or surgery, but it can be made with needle biopsy of the liver. No characteristic radiographic features of PNT have been described. Because PNT is such a rare condition, there are no specific treatment guidelines other than a recommendation for symptomatic management.

Focal nodular hyperplasia

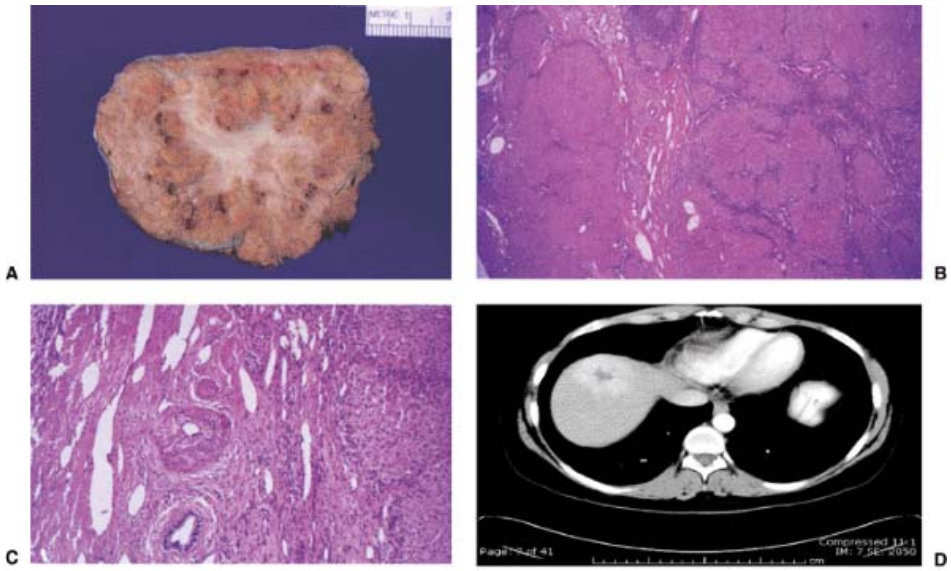
Focal nodular hyperplasia (FNH) is a benign, tumor-like lesion of the liver considered to be hyperplastic in origin rather than neoplastic. The exact frequency is not known, but large series of cases have been reported. FNH occurs predominantly in women but has also been found in men and children. It rarely causes clinical complications, but its importance lies mainly in being differentiated from hepatocellular adenoma and HCC. FNH classically occurs in noncirrhotic liver, but a recent report suggests that similar lesions may be seen in cirrhosis as well (12). This is discussed in detail in Chapter 42 of the book.

The cause of FNH is not known. It typically occurs in isolation but may be associated with

other liver diseases such as Budd-Chiari syndrome, pulmonary hypertension, primary sclerosing cholangitis, hemochromatosis, echinococcal cyst, and agenesis of the portal vein. Because of the female preponderance of this condition (female-to-male ratio approximately 8: 1), a link with oral contraceptive use has been postulated. However, Mathieu et al. (13), in a study in which the subjects were 216 women with FNH, examined tumors in patients taking oral contraceptives and those not taking these agents. The investigators found that neither the size nor the number of FNH lesions was influenced by oral contraceptive use. A more recent study identified an odds ratio of 2.8 for using oral contraceptives at any point of time among patients with FNH compared to controls (14). FNH may be associated with other vascular conditions affecting the liver, such as hemangioma and hereditary hemorrhagic telangiectasia (15,16).

In a study of the cases of 168 patients with FNH, investigators examined the gross and microscopic pathologic features of this condition (17). In 76% of patients, the mass was solitary and more often located in the right lobe than the left. In 21% of patients, between 2 and 5 nodules were found, whereas in the other 3% of cases between 15 and 30 nodules were detected. The lesions ranged in size from 1 mm to 19 cm in diameter. In total, 64% of masses were smaller than 5 cm in diameter. A central scar was visible on gross examination of 138 of 305 lesions (45%), and a large vascular pedicle was found in the periphery of 22 lesions (7%) (Fig. 43.4). Microscopic examination showed that all the lesions consisted of nodular hyperplastic parenchyma partially or completely surrounded by fibrous septae. Additional changes such as periodic acid–Schiff positive globules and Mallorys hyaline were sometimes found in hepatocytes within the lesion. A central scar was found on light microscopic examination of 153 lesions, and the lesions always contained malformed blood vessels of varying caliber. Histologic cholestasis was found in 36 cases, and some patients had hepatocellular steatosis. Portal tracts and terminal hepatic venules were not present.

FNH is often an incidental finding during abdominal surgery or imaging studies of the abdomen performed for another reason. Patients sometimes report abdominal pain or feel a right upper quadrant mass in the liver. The diagnosis is often suggested by certain characteristic radiographic features. The ideal technique is helical CT with images taken before infusion of contrast medium and in the arterial (early) and venous (late) phases. FNH typically becomes homogeneously enhanced with contrast material in the early phase, with rapid washout of contrast material such that the tumor becomes isodense to the liver on late portal venous and delayed images (18). On late portal venous and delayed images, enhancement of peripheral vascularity may be found. This gives the appearance of a rim around the tumor and corresponds to enlarged vessels or sinusoids at the periphery of the tumor. The central feeding artery may be seen in the arterial phase of the scan. On MRI, FNH is typically isointense or hypointense on T1-weighted images and slightly hyperintense or isointense on T2-weighted images (19). Most of these tumors are homogeneous, and the central scar appears hyperintense on T2-weighted images because of its vascularity. When gadolinium chelates are administered as contrast agents for MRI, a pattern identical to that seen on CT scans can be observed, that is, dramatic enhancement in the arterial phase followed by isointensity of the lesion during the portal venous phase. Technetium-99 sulfur colloid scans have been used to differentiate FNH from adenoma because the latter shows decreased uptake of radioisotope. This technology has largely been replaced by spiral CT and MRI.



▪ **Figure 43.4 A:** Macroscopic appearance of resected focal nodular hyperplasia. The typical central scar is evident. (Courtesy of Elizabeth M. Brunt, MD) **B:** Photomicrograph of focal nodular hyperplasia shows regenerating nodules of hepatocytes surrounding a central core that contains abnormal blood vessels and a bile duct. (Courtesy of Elizabeth M. Brunt, MD) **C:** Focal nodular hyperplasia with an abnormal, thick-walled artery adjacent to the center of the lesion. (Courtesy of Elizabeth M. Brunt, MD) **D:** Dynamic computed tomography scan of the liver showing focal nodular hyperplasia. Note hypervascular area in right lobe of the liver with a darker, central stellate scar.

Although the diagnosis of FNH can be made by the examination of a needle biopsy specimen of the liver, this approach is less than ideal because of sampling error. Needle biopsy samples only a small portion of the lesion. The specimen may contain only a group of hepatocytes that cannot be reliably differentiated from hepatocellular adenoma. Furthermore, the presence of fibrous septae within the lesion may give the appearance of cirrhosis (referred to as *pseudocirrhosis*). Therefore, if the results of radiographic studies are convincing and the patient has no symptoms, no further testing is needed. However, if there is uncertainty about the diagnosis, surgical resection or large surgical biopsy may be needed for accurate diagnosis of FNH (Table 43.3). A recent study suggests that application of a rigorous scoring system to liver biopsy specimens can aid in the accurate diagnosis of FNH (20).

Table 43.3. Comparison of Hepatocellular Adenoma and Focal Nodular Hyperplasia

Feature	Adenoma	Focal nodular hyperplasia
Sex	Female	Female
Oral contraceptive use	Strong association	Questionable
Symptoms	Occasional	Rare
Multiple	12%–30%	Approximately 30%

Central arterial scar	No	Yes
Treatment	Resection	Resection only if symptomatic

Adapted from Rodes J, Sherlock S. Focal nodular hyperplasia in a young female. *J Hepatol* 1998;29:1005–1009, with permission.

FNH rarely results in clinically important consequences or complications. Specifically, the risk of bleeding or rupture is very low, as is the risk of development of malignant changes. Few longitudinal studies have been done, but in one small series of 18 cases of FNH, the volume remained stable in 6, decreased in 10, and increased in 2 (21). Usually, no specific treatment is needed, but surgical resection is curative if the patient has symptoms. Resection is often performed in case of diagnostic uncertainty.

Unusual forms of focal nodular hyperplasia

The International Working Party has designated multiple FNH syndrome a distinct syndrome consisting of two or more FNH lesions in association with hemangioma of the liver, vascular malformations of the central nervous system, or tumors of meningeal or astrocytic origin (1). Of course, FNH is multiple in approximately 30% of cases, but most of these do not have the other features associated with this syndrome.

Nguyen et al. (17) found that approximately 20% of cases of FNH are *nonclassical*. This term refers to lesions lacking some of the typical histologic features, such as malformed vessels, a central scar, or abnormal nodular architecture. Notwithstanding these histologic differences, these cases were clinically identical to those with classic features.

Another variant of FNH is the telangiectatic form, which differs from typical lesions by the absence of a central scar and lack of architectural nodular distortion. Instead, there is sinusoidal prominence with associated hepatic plate atrophy. Telangietactic FNH (TFNH) is more likely to be monoclonal in origin than is classical FNH and is more likely to be multiple and perhaps even more likely to result in hemorrhage than typical FNH, making it more like adenoma (22,23).

Nodular Neoplasms

Hepatocellular adenoma

Hepatocellular adenoma, a benign neoplasm of hepatocytes, is described in detail in Chapter 42. It certainly manifests as a nodular condition of the liver that is usually solitary but is at times multiple. Adenoma nearly always occurs in women and only in those men taking sex hormones. Indeed, adenoma was an exceedingly rare tumor before the introduction of oral contraceptives. An important recent finding has been the genetic alterations in hepatocellular adenomas. Bilallelic mutations of the *TCF1* gene coding for the hepatocyte nuclear factor 1α (HNF1α) were identified in 60% of patients with adenoma (24). The Wnt pathway, also activated in approximately 25% of patients with HCC, seems to be activated in at least some cases of adenoma (25).

Although it can be detected incidentally while asymptomatic, adenoma often manifests as abdominal pain and can be complicated by hemorrhage or rupture. Adenoma can sometimes be difficult to distinguish from well-differentiated HCC, and there are reports of cancer developing within adenoma, whereas development of malignancy is exceedingly rare in case of FNH. Adenoma tends to grow, particularly in the presence of pregnancy or with continued

use of oral contraceptives.

It has been found that adenoma occurring in patients taking estrogens tends to shrink when these drugs are stopped. Adenoma occurring during pregnancy also tends to recede after the pregnancy is over. Surgical resection is usually recommended for adenoma to avoid the risk of rupture and hemorrhage. It may also be difficult to diagnose adenoma with confidence on the basis of radiologic findings on and needle biopsy, and with examination of the resected lesion is usually more conclusive.

Adenomatosis of the liver refers to the presence of more than four adenomata within the same liver and may have been confused at some time with NRH. The association with oral contraceptive use does not seem to be as strong with adenomatosis as it is with simple hepatic adenoma. Grazioli et al. (26) reported on a series of 15 patients with more than 10 adenomata and no history of glycogen storage disease or anabolic steroid use. Only one of the patients was of male gender but all were adults. Five of the 14 women gave a history of oral contraceptive use. Most of the patients had abdominal pain and hepatomegaly. In all patients the adenomata increased in size over time, and in two cases HCC developed. Four of the 15 patients underwent hepatic resection, and 5 underwent liver transplantation.

Comparison of hepatocellular adenoma and focal nodular hyperplasia

Hepatocellular adenoma is sometimes difficult to differentiate from FNH. The two conditions are compared in Table 43.3. Briefly, although FNH has a slight female preponderance, a link with female hormones in general and oral contraceptive use in particular has been difficult to prove, whereas these associations are very striking for adenoma. Whereas adenoma carries substantial risk of being complicated by hemorrhage and rupture, clinical complications of FNH are rare. Radiologic techniques to differentiate adenoma from FNH have improved considerably. Diagnosis of FNH relies on detection of the central scar, which is usually present and contains abnormal blood vessels. This central scar can be detected with ultrasonography, contrast-enhanced CT, MRI, and even angiography, whereas adenoma shows only nonspecific contrast enhancement and may be inhomogeneous because of the presence of hemorrhage or necrosis. Technetium-99 sulfur colloid liver scanning has also been suggested for differentiating these two liver tumors. This type of scan shows hepatocellular adenoma as an area of decreased or absent uptake in all patients. However, Herman et al. (27) found that approximately 60% of patients with FNH had a sulfur colloid scan showing decreased or absent uptake. Molecular analyses have shown β-catenin mutations in hepatic adenoma but not in FNH (28).

Hepatocellular carcinoma

HCC is a malignant neoplasm of hepatocytes and is described in Chapter 44. HCC has been described as

occurring in association with several of the nodular lesions described earlier, including NRH, dysplastic nodules, hepatocellular adenoma, and FNH. The link between HCC and NRH or FNH probably involves compromise of blood supply to the liver because of the presence of the malignant tumor, which causes direct pressure or microvascular invasion, whereas dysplastic nodules and adenoma may give rise to HCC.

Diagnostic Approach to Nodular Diseases of the Liver

In dealing with a patient with an unknown solid hepatic lesion, it is critical to determine whether the patient has underlying liver disease, and in particular whether cirrhosis is present. This involves serologic testing for various causes of liver disease and may even need liver biopsy away from the area of concern. If cirrhosis is present, HCC or dysplastic nodules are very likely to be found, whereas if there is not significant liver disease, FNH or adenoma should be considered. Imaging techniques have improved dramatically over the last decade and often allow excellent characterization of nodular lesions. Multiphasic CT scans using up-to-date equipment is capable of showing vascularity of hepatic tumors and nodules in great detail. Advances in diagnostic ultrasonography with color Doppler or the use of contrast agents may be useful in characterizing hepatic lesions (29,30). However, the greatest

advances appear to have occurred with MRI, and recent studies have demonstrated accurate differentiation of FNH from hepatic adenoma using contrast-enhanced MRI or distinction between adenoma or HCC and "nonsurgical" lesions such as FNH or regenerative nodules, although Krinsky and Israel found that dysplastic and nondysplastic nodules still cannot be separated (31,32,33).

Although needle biopsy of nodular hepatic lesions such as FNH may be difficult to interpret, there appears to be a recent impetus toward needle biopsy of lesions in cirrhotic livers in particular. Therefore, Bolondi et al. (34) have pointed out that a substantial number of nodules in cirrhosis are hypovascular, including HCC. Their observations call into question the validity of the noninvasive criteria for diagnosis of HCC. The accuracy and safety of ultrasonography-guided biopsy of these lesions has been documented (35).

Cystic Diseases of the Liver

Cystic diseases of the liver represent three groups of hepatic disorders that share the clinical feature of abnormal fluid-filled spaces in the liver and biliary tree. The first and largest group is fibrocystic diseases of the liver and biliary tree (Fig. 43.5). They are related hepatic disorders characterized by overgrowth of biliary epithelium leading to the production of fluid-filled dilated spaces, formation of portal fibrosis, and development of embryonic ductal plate malformations. The lesions result from malformations in different portions of the developing biliary tree and include polycystic liver disease (PCLD), simple hepatic cysts, congenital hepatic fibrosis (CHF), von Meyenburg complexes, and choledochal cysts (Table 43.4). The second group of disorders results from congenital defects of the embryonic foregut and includes cystadenoma and cystadenocarcinoma. The last disorder mimics the other cystic liver diseases and results from infection with the parasites *Echinococcus granulosus* or *Echinococcus multilocularis*.

Table 43.4. Bile Duct Segment Associated with Fibrocystic Disease of the Liver and Biliary Tract

Fibrocystic disease	Associated bile duct
Choledochal cysts	Common hepatic
Caroli's disease	Segmental area
Congenital hepatic fibrosis	Interlobular
Polycystic liver disease	Intralobular
Simple cysts	Intralobular

Fibrocystic Liver Disease

Polycystic liver disease

PCLD is a rare disease in which multiple cysts develop within the parenchyma of the liver. It is generally defined as the presence of four or more thin-walled cysts within the hepatic parenchyma because simple hepatic cysts are commonly characterized by one, two, or three cysts (36). PCLD occurs both in association with autosomal dominant polycystic kidney disease (ADPKD) and in isolation.

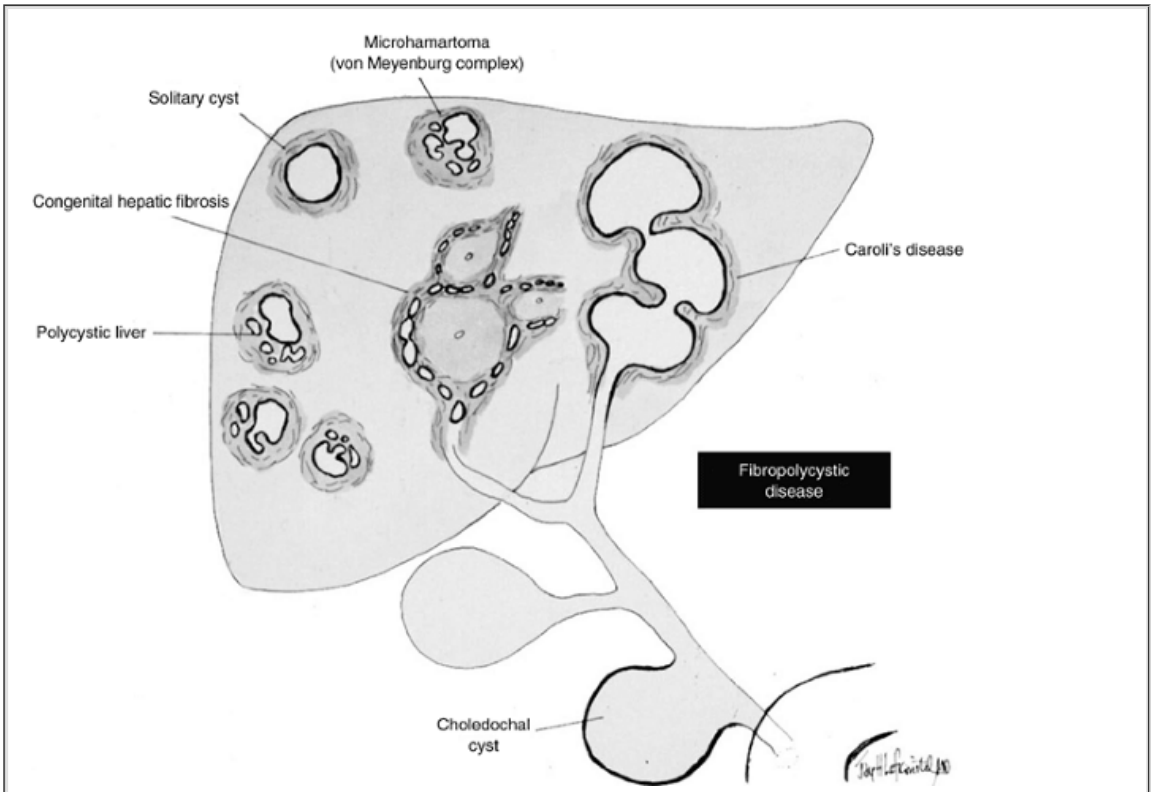
Epidemiology and course of disease

The true prevalence of PCLD is not known. Results in autopsy series suggest that the

prevalence ranges from 0.05% to 0.13% (37,38). The prevalence of multiple liver cysts in patients with ADPKD is 45% to 68% (38). Cyst prevalence in ADPKD increases from approximately 24% in the third decade of life to 80% in the sixth decade of life (39). The association of PCLD with ADPKD occurs in 16% to 93% of patients (40,41). This range is wide because the prevalence of PCLD in ADPKD depends on age and the amount of renal dysfunction (42).

When occurring with ADPKD, cysts generally begin to form in the liver after the onset of puberty. Although the proportion of men and women with liver cysts and ADPKD is the same, women tend to have both more and larger cysts (43). Cyst formation is thought to be

associated with estrogen exposure because the number and size of cysts correlate with number of pregnancies, use of oral contraceptives, and use of female hormone replacement therapy (43). Clinically relevant polycystic disease also correlates with advancing age, severity of renal cystic disease, and presence of renal dysfunction (42).



▪ **Figure 43.5** Diagram showing the relations between the fibrocystic diseases of the liver and biliary tract. (Artwork courtesy of Jay H. Lefkowitch, MD)

There are few published data on the frequency of symptomatic PCLD associated with ADPKD. Descriptions of symptomatic complications of PCLD are most often published in the form of case reports or reports of small series of patients over the course of many years. Most authors indicate that PCLD is predominantly a silent disease. The frequency of symptoms may be increasing because of the success of hemodialysis and renal transplantation; larger numbers of older patients with renal insufficiency are likely to have more and larger cysts within the liver. One report indicated that as much as 10% of the mortality among patients with ADPKD who undergo hemodialysis may be related to PCLD (44).

There is even less understanding of the epidemiology and course of isolated PCLD. Reports of old autopsy and surgical series suggest that multiple hepatic cysts may be present in the absence of renal cysts in as many as 50% of all patients with multiple hepatic cysts (45). This is probably an overestimation. Results of a more recent autopsy series suggested that only 7% of patients with PCLD did not have associated renal cysts (38). Several kindreds of

patients have been found to have multiple cysts in their livers and no evidence of ADPKD (37,46,47,48). Similar to ADPKD-associated PCLD, isolated PCLD is more severe in women and more severe disease is associated with pregnancy (49). Many patients are likely asymptomatic with clinically silent disease (49). Because these series represent small numbers of patients, there is no clear understanding of the course of isolated PCLD.

Genetics and molecular biology

ADPKD is one of the most common genetic defects, with a disease frequency of 1:1,000 in the white population (50). There are two genes associated with ADPKD—*PKD-1* and *PKD-2* (51). *PKD-1* is located on the short arm of chromosome 16 and is responsible for approximately 85% of cases of ADPKD (51). PKD-2 is located on the long arm of chromosome 4 and is responsible for about 15% of ADPKD (52). There are numerous mutations known for the *PKD-1* and *PKD-2* genes. The gene products, polycystin-1 and polycystin-2, are transmembranous glycoproteins that are thought to be involved in cell–cell or cell–matrix interactions. Polycystin-1 appears to interact with a G-protein signaling pathway that modulates calcium channels (52). Polycystin-2 is an integral membrane protein with the characteristics of a cation channel (52). Polycystin-1 and polycystin-2 complex in the cell membrane and localize in the primary cilium (52). The primary cilium is a microtubule-based cellular structure found on the luminal surface of epithelium, including biliary and renal tubular epithelium, which acts as a flow sensor and regulates Ca^{2+} influx. Loss of flow sensing has been hypothesized to lead to dedifferentiation, cell proliferation, and loss of restriction in tubule size, resulting in cyst formation (53). Although the genetic inheritance pattern is autosomal dominant, a cellular recessive two-hit model has been proposed. A germline mutation in *PKD-1* or *PKD-2* is not sufficient to produce disease, but a second somatic mutation in the functional *PKD* gene triggers monoclonally derived cyst formation (54,55).

Recent progress has been made in understanding the genetic basis of isolated PCLD. Reynolds et al. studied two large kindreds with autosomal dominant isolated PCLD and showed linkage to a putative causative gene on the long arm of chromosome 19 (48). Drenth et al. and Li et al. identified that mutations in PRKCSH are responsible for some cases of isolated PCLD (56,57). PRKCSH has been previously identified as protein kinase C substrate 80K-H, but the function of the protein product, hepatocystin, is unclear. Hepatocystin can function as the noncatalytic β-subunit of glucosidase II, which plays a major role in the regulation of proper folding and maturation of glycoproteins. PKD-1 and PKD-2 are glycoproteins, so it has been proposed that mechanistically there could be a link between ADPKD-associated PCLD and isolated PCLD if defective glycosylation from mutant glucosidase II results in improper functioning of polycystins (57,58). There is a preliminary report that mutations in SEC63 are associated with isolated PCLD in persons who have normal PRKCSH (58). SEC63 is also involved in the processing of integral and secreted proteins as a part of the multicomponent translocon involved in protein translocation (58). A similar two-hit model of ADPKD-associated PCLD has been proposed for isolated PCLD.

Pathology

The fluid-filled cysts of PCLD are usually scattered throughout the liver, although they can be present in only one lobe (Fig. 43.6). The cysts vary in size from less than 1 cm to more than 10 cm in diameter and cause massive enlargement of the liver. Microscopically, the cysts are close to or actually within the portal tracts (59). The cyst cavities are lined by flat or cuboidal epithelium, the presence of which is often associated with biliary microhamartoma (60). Fluid within the cysts is consistent with the bile salt–independent fraction of bile and increases with secretin stimulation. Therefore, the cysts are lined with biliary-type epithelium (61) and are hypothesized to form from the dilatation of biliary microhamartomata (39,60). The cysts may also arise from peribiliary glands (49). During their development the cysts become disconnected from the biliary tract (60). It is not clear whether the biliary microhamartomata are congenital or develop as the patient ages (60).

Clinical features

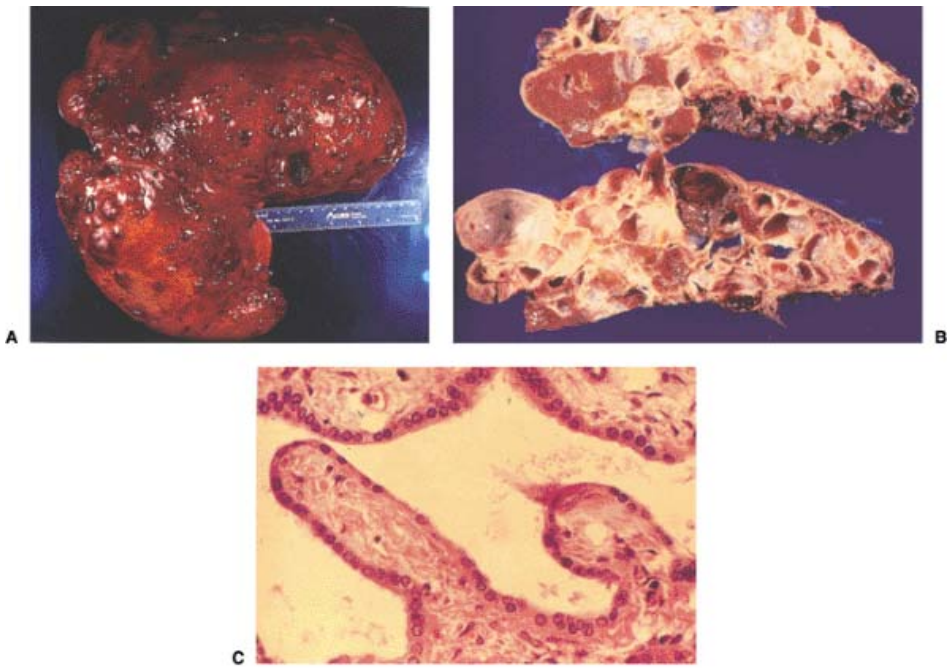
The symptoms of isolated and ADPKD-associated PCLD are most often caused by the

mechanical effect of the enlarged cystic liver. Most cases are asymptomatic (49). Some cases of PCLD may manifest as vague abdominal fullness or a palpable abdominal mass. Patients who underwent surgical intervention have been reported to have the following symptoms: Abdominal pain, abdominal distension, early satiety, fatigue, orthopnea, jaundice, ascites, and variceal bleeding (62). The right upper quadrant pain and shortness of breath experienced by patients with both PCLD and ADPKD correlate with larger liver volumes and not with kidney volume (63). Patients generally have preserved hepatic function on the basis of galactose elimination capacity and antipyrine clearance (62). Total hepatocyte volume remains normal, according to calculations of hepatic mass from CT images (42) (Fig. 43.7). Results of liver function tests are either normal or show a cholestatic pattern with usually mild elevations in alkaline phosphatase and total bilirubin levels. Serum albumin levels can be mildly depressed and prothrombin time elevated because of poor nutrition (49,62).

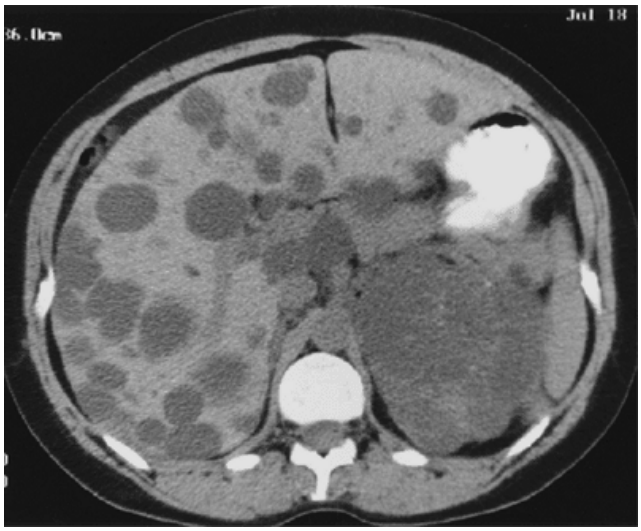
Complications of polycystic liver disease

The development of portal hypertension in PCLD is rare. There are 19 case reports of variceal bleeding related to PCLD in the literature (64). The pathophysiology of the development of varices is not clear. Some patients seem to have hepatic venous and or inferior

vena caval obstruction from direct compression of the cysts. Others may have associated CHF. Treatment involves obliteration of varices by means of repeated band ligation. Pharmacologic intervention with β-blockers or somatostatin analogs can also be attempted. TIPS is generally technically unfeasible because of the large cysts. Surgical debulking of the cysts can lead to relief of venous outflow tract obstruction and, therefore, of portal hypertension in some patients (62). Surgical portocaval shunts can be helpful. Liver transplantation can be considered in cases of refractory disease.



▪ **Figure 43.6 A:** Polycystic liver with massive enlargement of the liver. **B:** Cut surface of a portion of a polycystic liver containing numerous cyst pockets. (Courtesy of Elizabeth M. Brunt, MD) **C:** Photomicrograph of polycystic liver stained with hematoxylin and eosin showing cuboidal cyst lining. (Courtesy of Elizabeth M. Brunt, MD)



▪ **Figure 43.7** Abdominal computed tomography scan shows diffuse cystic involvement by polycystic liver disease.

Hepatic venous outflow tract obstruction has been reported in small case series (65). These patients have a history of PCLD and new development of ascites. Hepatic function is usually preserved, and the ascitic fluid has a high protein content. Findings on CT or MRI may suggest hepatic venous outflow tract obstruction, which can be confirmed with hepatic venography. Abdominal surgery may precipitate hepatic venous outflow tract obstruction. If there is no evidence of thrombus in the hepatic veins or vena cava, percutaneous or surgical treatment can be directed at the obstructing cyst. If thrombosis of vessels or the presence of multiple small cysts is the cause, surgical portosystemic shunting or liver transplantation should be considered. Transplantation should probably be reserved for patients with evidence of hepatic decompensation. Patients should be screened for hypercoagulable states with the appropriate laboratory studies.

Obstructive jaundice is a rare complication of PCLD, being described only in case reports. It results from direct obstruction of the biliary tree by a cyst. Endoscopic retrograde cholangiopancreatography (ERCP) is helpful to rule out other causes of biliary obstruction and to help identify the culprit cyst, usually with the aid of abdominal CT scan (66). Therapy can be with percutaneous drainage and sclerosis or with surgery.

Infection of hepatic cysts is a rare complication of PCLD. It occurs in 1% to 3% of patients with PCLD and ADPKD and appears to be more common in patients with renal failure (44). Most patients have fever and right upper quadrant or right flank pain (44). Leukocytosis or left shift is usually present, but results of liver function tests have a cholestatic pattern in fewer than one half of patients (44). Imaging with CT, ultrasonography, or MRI usually shows either a thickened cyst wall or different density of the cyst fluid (44). If there is doubt, an indium leukocyte scan appears to be more sensitive than a gallium scan in the diagnosis of cyst infection (44). Results of the culture of cyst fluid are almost always positive for single bacterial organisms, suggesting a hematogenous route of infection (44). Treatment is with percutaneous or surgical drainage and intravenous broad-spectrum antibiotics.

Both ADPKD-associated PCLD and isolated PCLD are associated with the development of intracranial aneurysms (67,68). Autopsy series show that 20% of patients with ADPKD have associated intracranial aneurysms (68). Screening for aneurysms is probably prudent before considering anticoagulation or surgical therapy.

Treatment

The method of management of liver cysts depends on the size, number, and location of the

cysts and on the clinical manifestations. Surgical options include both open and laparoscopic fenestration (deroofing) of the cyst, hepatic resection and cyst fenestration, and orthotopic liver transplantation (OLT). Percutaneous drainage has no role in treatment because of the almost universal recurrence rate, but drainage followed by addition of a sclerosing agent may be an alternative. Patients with one or a few cysts can be treated with laparoscopic fenestration or percutaneous drainage with sclerosis. The open surgical approach with cyst fenestration is more appropriate for larger numbers of and deeper cysts. Some surgeons have advocated open surgery with hepatic resection and cyst fenestration for patients with massive, highly symptomatic PCLD (62). This procedure is most appropriate for patients with multiple cysts and areas of parenchymal sparing. OLT is reserved for patients with massive diffuse bilobar disease, who are homebound and unable to perform activities of daily living, or who have evidence of hepatic failure (69).

Table 43.5. Selected Series of Patients who Underwent Open or Laparoscopic Surgery for Polycystic Liver Disease: Morbidity, Mortality, and Recurrence of Symptoms

Study	No. of patients	Operation	Mean follow-up period (y)	Morbidity (%)	Mortality (%)	Rate of recurrent symptoms (%)
Que (1995) (62)	30	R/F	2.7	58	3	3
Martin (1998) (69a)	6	OF	8	40	0	20
	9	R	0.75	67	0	0
	7	LF	3.1	29	0	71
Gigot (1997) (69b)	9	OF	4.2	56	0	11
	1	LF	4.7	0	0	0
Katkhouda (1999) (69c)	8	LF	ns	38	0	13
Kabbej (1996) (69d)	16	LF	2.2	63	0	73
Newman (1990) (69e)	9	OF	1.4	56	11	0
Soravia (1995) (69f)	10	OF	ns	20	10	30

R, resection; F, fenestration; O, open; L, laparoscopic; ns, not stated.

Table 43.5 lists selected series of both open and laparoscopic surgical results in the management of PCLD. In general, laparoscopic techniques have lower morbidity but higher rates of recurrence. Hepatic resection with cyst fenestration is the most effective technique in preventing recurrence, but it carries a high morbidity. Recurrence of symptoms is usually not caused by new cysts but by enlargement of the remaining cysts. Common postoperative complications include massive hemorrhage, biliary leaks, ascites, and infection. Careful patient selection is critical, especially with regard to the certainty that the symptoms are related to the liver cysts (70). If there is doubt, consideration should be given to percutaneous cyst aspiration to assess clinical response.

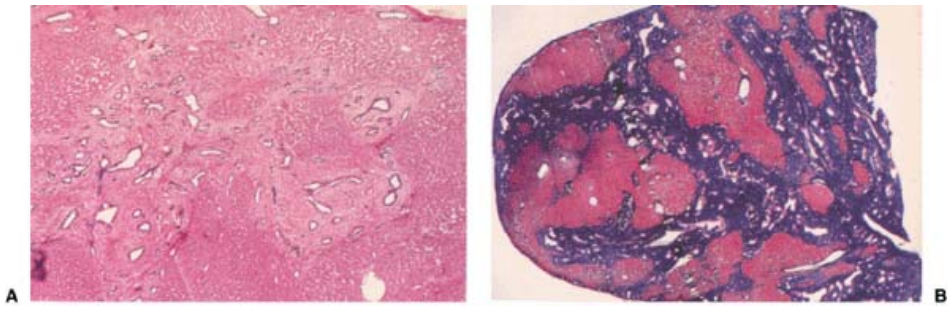
Several small series with limited follow-up evaluation have shown successful sclerosis of hepatic cysts with alcohol and minocycline hydrochloride. This technique is generally more successful for the management

of simple hepatic cysts than as therapy for PCLD. Complications include abdominal pain, fever, and ethanol intoxication. Care must be taken to avoid sclerosis of cysts that communicate with the biliary tree.

There are 76 case reports of patients who have been have undergone liver transplantation for highly symptomatic diffuse small cysts (71). Symptom relief was nearly universal among the surviving patients. The outcome of OLT is equal to or better than that of OLT performed for other typical indications. Previous attempts at surgery may increase the risk of perioperative complications. Earlier series typically performed liver and kidney transplantation, but more recent series suggest that combined transplantation is only necessary if significant renal dysfunction is present.

Simple hepatic cysts

Simple hepatic cysts are thought to result from congenital defects of intrahepatic bile ducts. They are lined with biliary-type epithelium but do not generally connect with the biliary tree. These cysts are estimated to have an incidence of 2.5% among the general population, with increased frequency with advancing age (36). They are most often asymptomatic incidental findings on abdominal imaging. When symptomatic, simple hepatic cysts can produce the same range of symptoms and complications as PCLD, although less frequently. Simple hepatic cysts can be differentiated from PCLD by lack of an autosomal dominant inheritance pattern, lack of associated renal cysts, and smaller numbers of cysts, usually less than four (48). Imaging with ultrasonography, CT, or MRI usually provides enough information for a diagnosis. On imaging the cysts appear as thin, smooth-walled anechoic masses or water densities. Any septations or papillary projections should raise suspicion for cystadenoma or cystadenocarcinoma Mergo and Ros (1998) (71a). Management of symptomatic cysts is by percutaneous sclerosis, open surgical or laparoscopic fenestration, or resection. Percutaneous treatment with aspiration followed by alcohol or another sclerosing agent is successful in most cases, and the recurrence rate is low. Open and laparoscopic fenestration can be performed with similar success but higher morbidity.



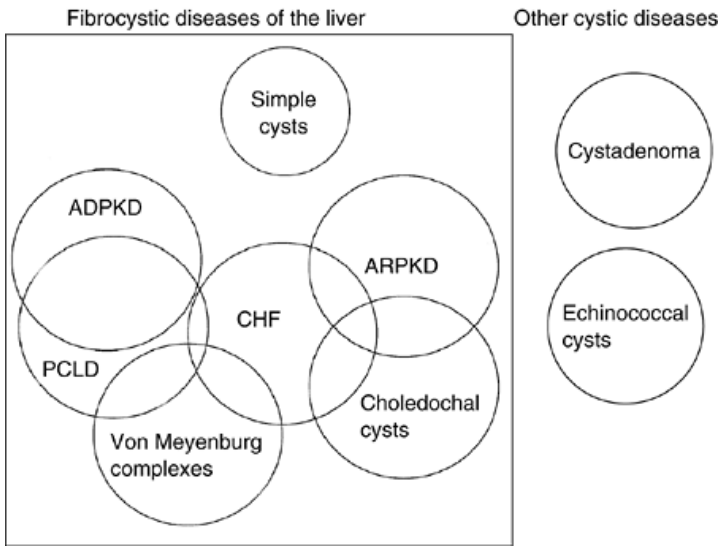
▪ **Figure 43.8 A:** Congenital hepatic fibrosis. Photomicrograph of the liver showing bands of scar with otherwise intact hepatic architecture and bile ducts in the scar lined by cuboidal epithelium (hematoxylin and eosin stain). (Courtesy of Elizabeth M. Brunt, MD) **B:** Congenital hepatic fibrosis. Photomicrograph showing highlighting bands of scar with otherwise intact hepatic architecture (Masson trichrome stain). (Courtesy of Elizabeth M. Brunt, MD)

Congenital hepatic fibrosis

CHF is a rare disorder that results in fibrous destruction of interlobular bile ducts. The liver shows fibrous enlargement of the portal tracts; portal–portal bridging forms thick bands of scar that contain abnormal bile ducts. Normal-appearing cuboidal cells line the bile ducts in the scar (Fig. 43.8). CHF has been reported in association with other fibrocystic liver diseases, including adult PCLD, Caroli's disease, choledochal cysts, and von Meyenburg complexes (Fig. 43.9). CHF is most commonly associated with autosomal recessive polycystic kidney disease (ARPKD). It is not known whether the genetic defect in ARPKD is responsible for CHF. At least one half and probably more of patients with CHF have associated renal disease. CHF also has been associated with a variety of rare pediatric syndromes, including renal dysplasia; nephronophthisis; Joubert's syndrome; *c*erebellar vermis hypoplasia, *o*ligophrenia, congenital *a*taxia, *c*oloboma, *h*epatic fibrocirrhosis (COACH) syndrome; Meckel's syndrome type 1, Jeune's syndrome, vaginal atresia, tuberous sclerosis, phosphomannose isomerase 1 deficiency, Ivemark's syndrome type 2, short-rib syndrome, and osteochondrodysplasia (72).

The incidence of CHF is unknown. Because of the many clinical associations and the variable clinical presentation, some investigators believe that CHF is not a single entity but a spectrum of diseases. The cause of CHF is unknown. Desmet hypothesized that at birth,

patients who will eventually have CHF have ductal plate malformations of the interlobular bile ducts. These immature bile ducts undergo destructive cholangitis that results in loss of bile duct profiles and formation of fibrous scar. The destructive cholangitis progresses at variable rates and may arrest, explaining the variety of clinical presentations of the disease (72). The cause of the associated portal hypertension has been hypothesized to be hypoplasia or compression of small branches of the portal vein (72).



▪ **Figure 43.9** Interrelation between cystic diseases of the liver. ADPKD, autosomal dominant polycystic kidney disease; PCLD, polycystic liver disease; CHF, congenital hepatic fibrosis; ARPKD, autosomal recessive polycystic kidney disease.

The four patterns of clinical presentation of CHF are portal hypertensive, cholangitic, portal hypertensive–cholangitic, and latent. Serum aminotransferase levels are usually normal, although the alkaline phosphatase level is sometimes elevated. These patients usually do not have cirrhosis and maintain normal hepatic lobular architecture with normal hepatic function. The diagnosis is based on findings at liver biopsy. Surgical biopsy may be needed to obtain sufficient liver for diagnosis.

The portal hypertensive presentation is most common, representing approximately 70% of cases (73). Portal hypertensive CHF usually manifests in childhood or young adulthood as complications of portal hypertension, especially variceal bleeding with intact hepatic function. Hepatosplenomegaly may be present. Wedged hepatic venous pressure is normal, a finding consistent with presinusoidal portal hypertension. Symptomatic portal hypertension has traditionally been managed with surgical placement of a portosystemic shunt. Development of hepatic encephalopathy after shunting is extremely rare, probably because hepatic function is intact. Endoscopic variceal band ligation may be used to control acute bleeding. Endoscopic obliteration of esophageal varices is at least theoretically useful, although it would entail long-term endoscopic surveillance. Long-term follow-up evaluation of surgical shunts has shown that 39% of patients have jaundice, 17% have recurrent bleeding, and 17% have hepatic encephalopathy (74).

The cholangitic form of CHF manifests as fever, right upper quadrant pain, and a cholestatic pattern in the levels of the liver-associated enzymes. At least initially, patients with cholangitic CHF do not have evidence of portal hypertension. Treatment is with intravenous antibiotics. Either ERCP or percutaneous transhepatic cholangiography (PTC) can be used to image the biliary tree and relieve obstruction. These patients often have associated Caroli's disease, which results in the cholangitic signs and symptoms. Repeated bouts of cholangitis can lead to secondary biliary cirrhosis and the need for liver transplantation. Patients should be treated for Caroli's disease as described later.

The portal hypertensive–cholangitic or mixed presentation is a combination of symptoms of portal hypertension and cholangitis. Treatment is based on the presence of the symptoms described earlier. The latent form is typically discovered either at autopsy or during evaluation for other problems. It is generally asymptomatic and therefore requires no treatment.

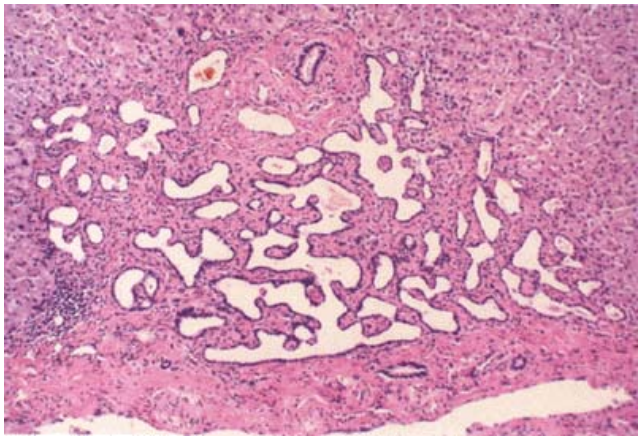
Rare reported associations with CHF include disease isolated to one lobe of the liver,

cholangiocarcinoma, ascites, and cavernous transformation of the portal vein.

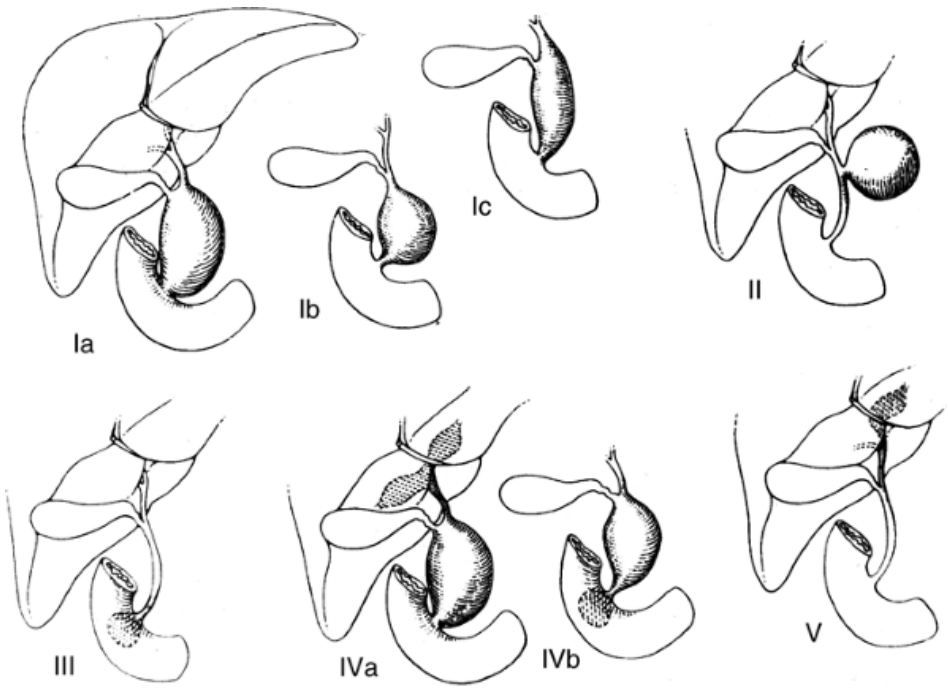
Von Meyenburg complexes

Von Meyenburg complexes, also called *biliary microhamartomata*, are usually incidental and asymptomatic

findings at liver biopsy. They are found in approximately 5% of adults and 1% of children in series of consecutive autopsies. The complexes consist of variable numbers of dilated bile ducts embedded in a fibrous stroma and occur in small groups adjacent to portal tracts (Fig. 43.10). The complexes may contain inspissated bile concretions and polypoid projections into the lumen. They are hypothesized to result from the fibrosis and occasionally from the involution of remnant ductal plate malformations of peripheral interlobular bile ducts (59). Von Meyenburg complexes are thought to be the origin of the cysts in PCLD. They are also found in association with Caroli's disease and CHF and in normal liver. There are rare reports of cholangiocarcinoma developing within a von Meyenburg complex.



▪ **Figure 43.10** Photomicrograph of the liver showing a typical von Meyenburg complex with a group of dilated bile ducts in fibrous stroma (hematoxylin and eosin stain). (Courtesy of Elizabeth M. Brunt, MD)



▪ **Figure 43.11** Drawing shows the Todani modification of the Alonso-Lej classification of choledochal cysts. (Adapted from Todani T, Watanabe Y, Narusue M, et al. Congenital bile duct cysts: classification, operative procedures, and review of thirty-seven cases including cancer arising from choledochal cyst. *Am J Surg* 1977;134:263–269, with permission.)

Choledochal cysts

Epidemiology, course of disease, and clinical features

Choledochal cysts are congenital dilatations of the intrahepatic and extrahepatic biliary tree. Vater and Elzer first described choledochal cysts in 1723 (75). The Todani modification of the Alonso-Lej classification of choledochal cysts is the system most often used for planning management of these cysts (76) (Fig. 43.11). The estimated incidence varies from 1 in 13,000 in Japan to 1 in 2 million in England (77,78). The frequency of diagnosis seems to be increasing, probably because of improvements in abdominal imaging (79). Type I cysts represent approximately 85% of most series (80). Type II cysts are rare, representing less than 2% of cases, and are sometimes called *common bile duct diverticulum* (81). Type III cysts or choledochoceles also are rare, representing approximately 2% of cases, although they may be more common in tertiary referral endoscopy centers (82). Type IV cysts represent the remaining approximately 10% of cases. Type V cysts are Caroli's disease.

The cysts usually manifest themselves in childhood but may be diagnosed in the prenatal period through late adulthood. Only approximately 20% of cases manifest themselves in adults (83). There is a female predominance (84). Neonates have jaundice and a palpable mass (84). In the first decade of life, ascending cholangitis is a more likely manifestation. The diagnosis is made

when ultrasonography, CT, hydroxy iminodiacetic acid (HIDA) imaging, endoscopic retrograde cholangiography, PTC, or magnetic resonance cholangiography shows characteristic dilatation of the biliary tree. Liver function tests may show elevated alkaline phosphatase, total bilirubin, and γ-glutamyl transpeptidase levels consistent with biliary obstruction (84). Manifestation in adulthood is often preceded by several years of vague right upper quadrant abdominal pain (85). At diagnosis the patient usually has a complication such as jaundice,

cholangitis, or pancreatitis (85). CHF is often associated with choledochal cysts, especially Caroli's disease.

Etiology

There are several theories about their etiology, although the true cause of choledochal cysts remains unclear. One theory suggests that failure of canalization of the fetal biliary tree results in obstruction and, later, dilatation of the common bile duct. Another theory suggests that obstruction occurs from anatomic kinking or external pressure on the developing bile ducts that results in cyst dilatation. Probably the most accepted theory was first proposed by Babbit, which stated that the high insertion of the common bile duct into the pancreatic duct leads to reflux of pancreatic juice into the biliary tree. This leakage causes weakening of the bile duct wall, inflammation, and fibrosis. The distal bile duct becomes obstructed and therefore results in proximal dilatation with cyst formation (86). There appears to be a high rate of anomalous pancreaticobiliary duct junction in the range of 90% in most series (87).

Treatment

Management of choledochal cysts is based on the type of cyst according to the Todani classification (Fig. 43.11). Some authors argue that the Todani classification scheme is misleading and overcomplicated (88). They propose a simplified naming system matched to the treatment and risk of future malignant transformation, which is described at the end of this section. The first premise of treatment is to obtain adequate drainage and antibiotic coverage for any patient with signs and symptoms of cholangitis. This may be achieved with PTC or ERCP.

The current standard of practice for type I and II choledochal cysts is complete cyst excision with anastomosis of the bifurcation of the hepatic ducts, common hepatic duct, or common bile duct with Roux-en-Y anastomosis to the jejunum or directly to the duodenum. Biliary enteric anastomosis at the bifurcation of the hepatic ducts appears to offer the advantage of a lower rate of late strictures (89). Cyst enterostomy is generally no longer performed because of the high rate of long-term complications, including anastomotic strictures that result in jaundice and cholangitis and late development of cholangiocarcinoma (85,90). Patients who have undergone previous cyst enterostomy and have late complications of surgery need complete cyst excision with Roux-en-Y hepaticojejunostomy. Patients with an asymptomatic cyst who are otherwise good surgical candidates should be considered for elective cyst excision and Roux-en-Y hepaticojejunostomy to prevent the development of cancer (85).

Type III choledochal cysts are also called *choledochoceles*. Patients have pancreatitis, obstructive jaundice, or biliary colic (91). They may have a history of cholecystectomy for acalculous cholecystitis with continued symptoms after surgery (91). The differential diagnosis includes papillary tumor, papillitis from an impacted stone, papillary fibrosis, pancreatitis, duodenal duplication, and submucosal contrast injection during ERCP (79). This broad differential diagnosis warrants cholangiography and biopsy to rule out tumors (73). Choledochoceles rarely develop into cancer, and therefore complete excision may not be mandatory (79,82). Large cysts should be excised either partially or completely and the ducts reanastomosed to the duodenum (91). Small choledochoceles can be managed with endoscopic sphincterotomy (79,92). Endoscopic pancreatic sphincterotomy may be necessary if the pancreatic duct does not drain well (82).

Type IVa choledochal cysts with both intrahepatic and extrahepatic components make total cyst excision difficult. Total excision of the extrahepatic cysts can usually be accomplished, and anastomosis with the enteric tract should be performed at the hilum to prevent a high rate of anastomotic strictures. Formation of a Hutson loop, which provides easy percutaneous access to the reconstructed biliary tree, has been advocated, especially if intrahepatic cysts are not resected (93). Partial hepatectomy, if technically feasible, has also been advocated for the removal of intrahepatic cysts because of malignant potential. The risk of biliary cancer in the retained intrahepatic cysts is not known but is probably low; only eight cases have been reported in the literature (94).

The presence of type V cysts, also known as *Caroli's disease*, results in multiple intrahepatic biliary cysts and makes treatment more difficult. Complete resection of biliary cysts is usually

impossible because of the location of the cysts. For this reason, conservative treatment of cholangitis with antibiotics is usually the initial choice. Rotating antibiotics to suppress episodes of cholangitis is advisable but is of unproven value. If the cysts are superficial and limited in number, hepatic resection can sometimes be curative. An alternative is to relieve obstruction with partial cyst excision and Roux-en-Y cyst jejunostomy. Liver transplantation is generally reserved for patients with hepatic failure. Prognosis is much worse for this type of choledochal cyst than for the others. Tsuchida et al. (95) found a

63% mortality in a survey of patients whose cases were reported in the literature.

Without therapy for choledochal cysts, biliary cancer, usually cholangiocarcinoma, develops in approximately 15% to 19% of patients older than 20 years and generally carries a dismal prognosis (85,90). The most common site of the cancer is the cyst wall, but as many as 40% of cases occur at other sites in the liver or pancreas, especially the gallbladder (81). For this reason and to prevent future gallbladder-related symptoms, cholecystectomy is usually performed during cyst surgery.

Complications include jaundice, pancreatitis, spontaneous cyst rupture, biliary cirrhosis, and signs of portal hypertension, including variceal bleeding and ascites (96). The postoperative rate of recurrence of symptoms can be as high as 32%, but the symptoms most often respond to conservative therapy with antibiotics (97).

The simplified system calls type I and IVa cysts "choledochal cysts" because they are variations of a single disease with dilatation of the extrahepatic biliary tree and varying levels of involvement of the intrahepatic tree. They represent the most common type of choledochal cysts, have the highest risk of malignant transformation, are associated with pancreaticobiliary maljunction, and require complete excision of the extrahepatic biliary tree with a high biliary enteric anastamosis. Type II cysts are rare diverticuli of the common bile duct that can be treated with simple excision of the cyst. Type III should be called *choledochoceles* because they are lined with duodenal mucosa, do not require complete excision, and rarely develop malignant transformation. Type V cysts should be called *Caroli's disease* and differ from the other types by affecting only the intrahepatic ducts, being associated with ductal plate malformation, having low malignant potential, and being associated with CHF and portal hypertension.

Cystic Neoplasms of the Liver

Cystadenoma

Cystadenoma is a benign tumor of the liver in which an epithelial layer surrounds a large, fluid-filled cyst in the hepatic parenchyma or rarely in the extrahepatic biliary tree. It represents less than 5% of cystic lesions of the liver. Cystadenoma is hypothesized to arise from a congenital defect of the bile ducts or gallbladder. It may form out of the rests of primitive foregut. It can be found in association with bile duct hamartoma.

Cystadenoma manifests itself any time from childhood onward, although 80% to 85% of cases occur among middle-aged women. The clinical manifestations are typically the result of compression of an expanding liver mass on adjacent structures. Approximately 70% of patients have epigastric or right upper quadrant pain, sometimes with radiation to the right shoulder. Approximately one half of patients have a palpable abdominal mass or increasing abdominal girth, and approximately one third of patients have compression of the biliary tree that leads to cholangitis or jaundice (98). Some patients have symptoms of gastric compression, such as nausea, vomiting, bloating, and anorexia. In some patients, the cysts are incidental findings on imaging of the abdomen. Extrahepatic cystadenoma typically manifests as abdominal pain and jaundice (99).

Complications are rare and have been reported to include sepsis, hemorrhage, and rupture. Transformation into malignant cystadenocarcinoma can occur and result in local invasion and occasional distant metastasis. Nearly all the reported cases of malignant transformation occur in patients who have the stromal layer.

The diagnosis of cystadenoma is suggested by the ultrasonographic findings of a multilocular anechoic fluid-filled cyst with thickened walls and multiple septations or papillary projections

(100). The cyst is usually located in the hepatic parenchyma, although extrahepatic cysts have been reported. There is usually no connection with the biliary tree. Levels of liver-associated enzymes are usually normal unless there is biliary obstruction. The serum level of cancer antigen 19-9 (CA19-9) may be mildly elevated, but levels of carcinoembryonic antigen (CA125) and α-fetoprotein are normal. Cyst fluid CA19-9 level is elevated, but aspiration carries a small risk of dissemination if carcinoma is present. The lesions should be differentiated from pyogenic abscess, amebic abscess, and echinococcal cyst on the basis of clinical manifestations and imaging findings.

Pathologic examination reveals cuboidal and columnar epithelial cells with vacuolizations lining the cysts. Usually adjacent to the epithelium is an area of spindle cell stroma (also called *mesenchymal stroma*) surrounded by a layer of collagenous connective tissue. Mesenchymal stroma are exclusively found in female patients and may represent a different form of the disease than those without stroma (101). The cyst fluid is usually mucinous, but it may contain bile or blood.

Treatment is complete surgical resection by means of either enucleation (excision of a mass with a thin layer of normal tissue) or anatomic resection. In the past, incomplete excision or drainage procedures led to a high recurrence rate and did not remove the risk of malignant transformation. There is a 50% rate of recurrence of extrahepatic cysts without sleeve resection with biliary enteric anastomosis (102).

Echinococcal Cysts

Hydatid disease caused by *Echinococcus granulosus* cystic echinococcosis is endemic in parts of western and southern Europe, the Middle East, northern Africa,

South and Central America, Russia, and China. The annual incidence ranges from less than 1 case to as many as 42 cases/100,000 persons in some regions (103). Humans are accidental hosts who ingest eggs from contaminated dogs that were usually infected from sheep. The liver is involved in one half to three fourths of cases. The cysts range from 1 to greater than 20 cm in size. Rarely, hydatid disease called *alveolar echinococcosis* can occur due to *Echinococcus multilocularis*. It is endemic in parts of North America, central Europe, and northern and central Eurasia. Incidence is generally less than 1/100,000 (103). Humans are accidental hosts who ingest eggs from foxes or forest rodents.

Diagnosis is based on a high index of clinical suspicion and findings on imaging with ultrasonography, CT or MRI. The initial symptoms are usually caused by complications of the cysts or physical compression of adjacent organs by the cysts. Symptoms such as epigastric or right upper quadrant pain, fatigue, fever, nausea, and dyspepsia may be present. Cyst rupture can produce jaundice, cholangitis, acute pancreatitis, and immune reactions such as anaphylaxis or asthma. Ultrasonography, CT, and MRI are sensitive and specific for hydatid disease, with cross-sectional imaging being superior in identifying the location of the cysts, including extrahepatic cysts, and type of cyst. Serologic tests, including an indirect hemagglutination test and enzyme-linked immunosorbent assay for antibodies to *Echinococcus* antigens are approximately 90% sensitive but have variable specificity because of cross-reaction with other parasites and may not distinguish cystic from alveolar disease.

There is controversy about the most appropriate treatment for cystic echinococcosis. Some advocate treatment even in asymptomatic patients to prevent complications such as rupture, infection, or anaphylaxis. Others have shown that small, nonsuperficial, and asymptomatic cysts can remain stable for more than 10 years. The choice of treatment depends on the type of cyst (usually defined by ultrasonographic appearance), the location of the cyst, the general medical status of the patient, and local experience. Because of its potential for aggressive infiltration of the liver and other organs and high mortality, radical surgical resection and at least 2 years of chemotherapy is the first choice of treatment for alveolar echinococcosis.

Open surgery with cyst removal or drainage and obliteration of the cyst cavity was until recently the primary mode of treatment for cystic echinococcosis. Although in the literature results of series of open surgical treatment are variable, they generally show a mortality ranging from 0% to 5%, morbidity from 8% to 25%, and long-term recurrence rates of 2% to 25%. Several series of laparoscopic cyst surgery have shown morbidity and mortality similar

to those of open surgery but they generally involve less difficult cyst types and locations. Any surgical or percutaneous therapy carries a risk of dissemination of daughter cysts into the abdomen, but modern techniques and experienced surgeons significantly limit the risk.

*P*uncture, *a*spiration, *i*ntroduction of protoscolicidal agent, and *r*easpiration (PAIR) is a percutaneous treatment developed by interventional radiologists in the mid-1980s. The connection of the cyst to the biliary tree is an absolute contraindication for this procedure, and only certain configurations of cysts are amenable to this treatment. Series of patients treated with PAIR and chemotherapy at experienced centers showed no mortality and less than 10% morbidity but the cysts were generally smaller, less complex, and amenable to a percutaneous approach. Long-term recurrence rates are not yet available but short-term results look promising.

Chemotherapy with benzimidazole compounds, mebendazole, or albendazole is generally reserved for patients not amenable to treatment by other means or as an adjunct to other treatment, especially PAIR (104). Chemotherapy has approximately a 30% cure rate, but up to 70% of cysts will reduce in size. Relapse rates are about 25%.

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Chapter 44

Hepatocellular Carcinoma

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Key Concepts

- Hepatocellular carcinoma (HCC) is a neoplasm that has well-defined risk factors, such as chronic viral infection and excessive alcohol intake. These induce chronic liver disease, that is, cirrhosis, and induce genetic damage, leading to cancer development.
- HCC is the leading cause of death in patients with cirrhosis.
- The sole option to reduce cancer-related death is to detect cancer at an early stage and apply effective therapy. Patients with cirrhosis who would be treated if diagnosed with liver cancer should enter screening protocols.
- Early detection of HCC should be based on hepatic ultrasonography every 6 months. Unfortunately, tumor marker determination lacks efficacy.
- Effective therapies for HCC with potential long-term cure include surgical resection, liver transplantation, and percutaneous ablation. Among palliative approaches, the sole approach with positive impact on survival is transarterial chemoembolization.
- Prevention of HCC should come from avoidance of risk factors by vaccination for the prevention of hepatitis B and maintenance of proper health standards and adequate lifestyle. Antiviral therapy may cure viral infection and hence prevent progression to cirrhosis and cancer.

Until recently, it was frequent to consider hepatocellular carcinoma (HCC) as a cancer with low incidence in the western world. However, recent data show that its incidence has increased in several western countries (1). In addition, cohort studies reveal that HCC is currently the leading cause of death in patients with cirrhosis (2,3). The feasibility of early detection associated with the availability of several effective therapies has permitted encouraging long-term survival after diagnosis, and as a result, the interest in all aspects related to diagnosis and treatment has sharply increased (4). In this sense, it has been recognized that hepatologists play a key role in the management of patients with HCC. They decide whether HCC surveillance is required in patients at risk and are responsible for disease staging and treatment indication. Among this, the most critical decision is the selection of candidates for liver transplantation and their management before and after surgery. In some countries, mostly in Asia, even surveillance and percutaneous treatment (i.e., alcohol injection and

radiofrequency ablation) are performed by hepatologists because they receive specific training to acquire the needed expertise.

In the present chapter we summarize the most relevant issues about epidemiology, diagnosis, and treatment of this neoplasm.

Epidemiology

Primary liver cancer is now the fifth most common cancer in the world and the third cause of cancer-related

mortality. More than half a million cases are diagnosed every year, there being major geographic differences in incidence. The annual incidence rates in eastern asia and sub-Saharan Africa exceed 15/100,000 inhabitants, while the figures are intermediate (between 5 and 15/100,000) in the Mediterranean basin and southern Europe and very low (<5/100,000) in northern Europe and America (1). Vaccination against hepatitis B virus (HBV) has induced a decrease in HCC incidence in countries where this virus is highly prevalent (5), whereas the contrary is true in areas where viral dissemination (mostly hepatitis C virus [HCV]) has occurred in the last decades (6). These data suggest that the geographic heterogeneity is related to differences in the exposure rate to risk factors and time of acquisition rather than to genetic predisposition. In this regard, studies in migrant populations have demonstrated that first-generation immigrants carry with them the high incidence of HCC that is present in their native countries, but in the subsequent generations the incidence decreases (7).

The age at which HCC appears varies according to gender, geographic area, and risk factors associated with cancer development. In high-risk countries with major HBV dissemination, the mean age at diagnosis is usually below 60 years, although it is not infrequent to observe HCC in childhood, thereby emphasizing the impact of viral exposures early in life (1). Contrarily, in intermediate- or low-incidence areas most cases appear beyond 60 years of age. In all areas, males have a higher prevalence than females, the gender ratio usually ranging between 2:1 and 4:1, and in most areas the age of occurrence in females is higher than that in males (1).

Risk Factors for Hepatocellular Carcinoma

Cirrhosis underlies HCC in more than 80% of the affected individuals (2,8). Therefore, any agent leading to chronic liver damage, and ultimately cirrhosis, should be seen as a risk factor for HCC. Obviously, the major causes of cirrhosis, and hence HCC, are HBV, HCV, and alcohol, but less prevalent conditions such as hemochromatosis, primary biliary cirrhosis, nonalcoholic steatohepatitis, and Wilson disease have been also associated with the development of HCC. The risk among those with cirrhosis increases in parallel with the impairment of liver function and is higher in males, patients older than 50 years, and subjects with increased α-fetoprotein (AFP) concentration (2). Pathologic characteristics such as increased proliferation, presence of dysplastic cells, or irregular regeneration have also been proposed as useful risk markers but are not fully validated.

Hepatitis B Virus

The evidence linking HBV with HCC is unquestionable (9). Active viral replication implies a higher risk, and long-standing active infection resulting in cirrhosis is the major event leading to increased risk (10,11). The incidence of HCC in

inactive HBV carriers without liver cirrhosis is less than 0.3% (8). The role of specific genotypes or mutations is not well established (12). HBV can be integrated into the host cellular genome and induce genetic damage. Deoxyribonucleic acid (DNA) integration in nontumoral cells in patients with HCC suggests that genomic integration and damage precede the development of tumor. Therefore, infection with HBV may be correlated with the emergence of HCC even in the absence of liver cirrhosis. In addition, some of the HBV proteins disrupt cellular functions (13) and favor neoplastic transformation, induce proliferation, and impede apoptosis (14).

Interestingly, occult HBV infection may become apparent if properly investigated by molecular techniques even in the absence of serologic markers of HBV (15). Identification of the HBV genome has been reported in liver tumors of patients who are HCV positive and HBsAg negative in the serum. The rate of occult infection in these patients can be as high as 63% (15). Finally, the implementation of vaccination against HBV has resulted in a significant decrease in the incidence of HCC (5), and this is the final proof of the importance of this virus in the genesis of HCC.

Hepatitis C Virus

The prevalence of HCV in HCC cohorts varies according to the penetration of the agent in the population of each geographical area. There is a single prospective population-based study of the risk of HCC in patients with hepatitis C (16). This study included 12,000 men and described a 20-fold increased risk of HCC in infected individuals, the figure being very close to the estimated risk obtained in a meta-analysis of 21 case–control studies (17). The risk is clearly related to the degree of liver damage induced by the virus (18). There are some case reports of healthy HCV carriers with HCC (19), but several cohort studies indicate that the incidence in patients with chronic hepatitis is low (below 1%) and that the risk increases sharply when cirrhosis is established (18). At this time, the annual incidence ranges between 2% and 8% (2). The transition from acute infection to cirrhosis may take 20 to 30 years. Some authors suggested the development of HCC even after a sustained response to interferon for the treatment of chronic hepatitis C (20).

Patients infected with human immunodeficiency virus (HIV) are now effectively treated with combined

regimes and, if coinfected with HCV, they present a faster evolution to cirrhosis (21) and, therefore, are at risk for the development of HCC. In fact, liver disease and/or HCC are the major causes of death in these patients (22).

Alcohol, Tobacco, and Coffee

A large proportion of patients with alcoholic cirrhosis are infected by HCV, and therefore, the risk-based studies before the recognition of this virus have surely overestimated the role of alcohol. The risk for HCC increases linearly beyond an estimated intake of 40 to 60 g/day of alcohol and also with the length of consumption and coinfection with HBV or HCV (23). On development of decompensated cirrhosis, the yearly incidence increases beyond 2%. Smoking slightly increases the oncogenic risk (24), whereas coffee consumption reduces the risk (25).

Pathogenesis

Active inflammation with oxidative damage is thought to be the key event leading to liver cancer, but the detailed molecular mechanisms are not known. Cumulative genetic changes occur and allow the appearance foci of high-grade dysplastic hepatocytes without overt malignant phenotype, which in one third of the cases may evolve into overt HCC after a follow-up of 5 years (26,27). Intense neoangiogenic activity accompanies this transition and results in enhanced blood supply derived from the hepatic artery that is known to permit radiologic characterization (28). The most frequently affected chromosomes are 1, 4, 8, 16, and 17, but none of them is abnormal in more than 60% of the cases (29). Downregulation of p53 is observed in up to 40% of the cases and G to T mutation reflects genetic damage due to aflatoxin intake.

Expression of several genes has been related to growth and dissemination, but none of them has been strongly validated to become part of the decision-making process in clinical practice.

Pathology

The appearance of HCC involves its transition from early to advanced stages (28). It is common to use the Edmondson and Steiner criteria to grade the degree of differentiation according to nuclear irregularity, hyperchromatism, and nuclear/cytoplasmic ratio. In HCC the cytoplasm shows fine granular eosinophils and may accumulate bile and fat, Mallory bodies, and α_1-antitrypsin globules. Fat deposition is seen in 30% to 40% of tumors approximately 1.5 cm in size and translates into increased echogenicity at ultrasonography (30). Immunostaining can recognize the presence of cytokeratins 7, 8, 18, and 19; carcinoembryonic antigen; AFP; and several other markers, but the findings are not sensitive or specific enough to be used to make the diagnosis. Malignant hepatocytes accumulate as thin (microtrabecular) or thick (macrotrabecular) layers separated by sinusoids that may contain Kupffer and stellate cells. Gross appearance may be described as expansive, infiltrative, and diffuse. The first type shows distinct margins and a surrounding reticulin pseudocapsule. No distinct margins are seen in the infiltrative type, and the diffuse type corresponds to a multinodular tumor that mimics a cirrhotic liver. Usually, HCC appears as a distinct nodule of varying size that increases together with the development of additional tumor sites first in the vicinity or in separate segments. Portal vein branches are invaded, and at late stages the entire liver may be occupied by malignant foci and the tumor spreads outside the liver (initially to lymph nodes and then to adrenal glands, lungs, and bone). The prevalence of portal vein invasion increases together with tumor size. Less than 20% of HCC smaller than 20 mm in size have microscopic vascular invasion, and recently, well-differentiated HCC without such an invasive profile and without minute satellite nodules has been named *very early HCC* or *carcinoma* in situ (31,32). This initial lesion still does not have increased vascularization and, currently, is only confidently diagnosed after resection.

Clinical Manifestations

Because most HCC cases appear in the setting of cirrhosis, a major part of the findings will be indistinguishable from the clinical picture observed in patients with advanced cirrhosis. Patients may present with jaundice, ascites, encephalopathy, or bleeding due to ruptured esophageal varices. Cancer-related symptoms such as abdominal pain or constitutional syndrome (e.g., weight loss,

anorexia, and malaise) reflect advanced tumor stage. Acute hemoperitoneum due to ruptured HCC or bone metastases is the first symptom in a minority of cases.

Advanced HCC is associated with increased bilirubin, alkaline phosphatase, and γ-glutamyl transpeptidase levels. Alanine transaminase (ALT)/aspartate transaminase (AST) concentration has no diagnostic value. Paraneoplastic manifestations include diarrhea and severe hypoglycemia, which in some cases are the most relevant concern. Other manifestations include hypercalcemia, sexual changes, polymyositis, thrombophlebitis, and skin rashes.

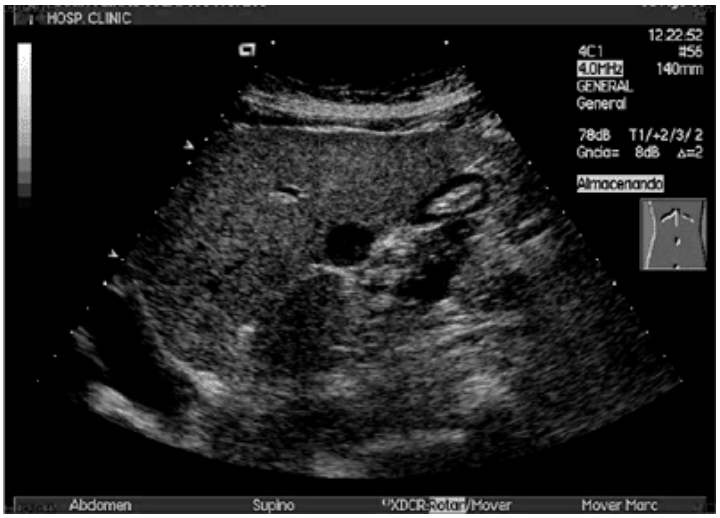
Diagnosis and Staging

Diagnosis at advanced stage, when cancer-related symptoms are present and a large mass can be recognized even by physical examination, is not difficult. The development of ultrasonography has permitted the detection of HCC at an asymptomatic stage, and its incorporation into the clinical workup of patients with known or suspected liver disease has sharply changed the diagnostic algorithm and prompted the establishment of early detection plans for HCC in the population at risk, that is, patients with cirrhosis. The efficacy of surveillance depends on the level of risk and on the availability of effective therapy. Hence, surveillance should be initiated on development of cirrhosis and be restricted to patients who would be treated if diagnosed with HCC. Therefore, screening should be limited to Child-Pugh A and B patients, while Child-Pugh C patients should be evaluated for liver transplantation and HCC in them could become a contraindication for the procedure. There is a single randomized controlled trial of surveillance versus no surveillance (33). It included more than 18,000 Chinese patients and has shown a survival benefit from a strategy based on a 6-monthly surveillance with AFP and ultrasonography. Several cohort studies have also shown that surveillance allows detection and diagnosis at an earlier stage and suggest an improvement in patients' survival.

AFP has very low sensitivity and slight increases may also be observed in patients with cirrhosis in the absence of malignancy (34,35). Therefore, it has no clinical role in screening and a very reduced role in the diagnosis, this probably being limited to patients with very advanced tumor stage in whom no treatment is provided. In these terminal patients, no imaging technique is needed after ultrasonography to confirm and stage the disease. Other tumor markers such as lectin-bound AFP (36), des-γ-carboxyprothrombin (37), or glypican (38) have been proposed to surpass the efficacy of AFP, but their clinical efficacy is yet to be unequivocally proved. Proteomic techniques may help identify new markers (39).

All these data indicate that surveillance programs should be based on regular ultrasonographic examination (Fig. 44.1). On the basis of the data on tumor volume–doubling time, most experts recommend patients with cirrhosis to be screened by ultrasonography every 6 months. It is important to note that increased risk does not mean faster tumor progression, and therefore, patients with cirrhosis who are at higher risk do not require screening at shorter periods. In the year 2000, the panel of experts of the European Association for the Study of the Liver (EASL) proposed an algorithm to diagnose nodules detected by ultrasonography that varied according to tumor size (2). Nodules less than 10 mm in size were considered not feasible to be confidently diagnosed and in some cases did not correspond to premalignant or malignant foci. Therefore, close follow-up was recommended, which is still valid. For nodules between 10 and 20

mm in size, the EASL recommended a positive result of the biopsy to establish the diagnosis, while for those larger than 20 mm a specific radiologic profile observed by two imaging techniques was considered to make the diagnosis if the patient was known to present with underlying cirrhosis. The improvement of dynamic imaging has allowed slight modification of the recommendations. Therefore, even in nodules between 1 and 2 cm in size the HCC diagnosis can be established if two dynamic imaging techniques (e.g., ultrasonography contrast, computed tomography [CT], and magnetic resonance imaging [MRI]) show that the nodule exhibits enhanced arterial uptake with contrast washout in the venous phase. In larger lesions, just one technique with a specific profile is enough to make the diagnosis; it is considered accurate enough. Therefore, biopsy of a nodule larger than 1 cm in size found on screening of a cirrhotic liver is needed if it does not show arterial enhancement with washout and if it is clearly not some other lesion, such as a hemangioma. It has to be noted that HCC nodules may appear echogenic because of the presence of fat in the cells, or may more usually be hypoechoic or even show a "target lesion" appearance (40). Obviously, a positive result of the biopsy confirms the diagnosis, but even if a nodule corresponds to an HCC, it might be negative for malignancy in up to 30% to 40% of cases. Accordingly, a negative result of the biopsy report does not confidently exclude malignancy.



▪ **Figure 44.1** A small nodule measuring less than 20 mm in size detected during ultrasonographic surveillance. It corresponds to an early hepatocellular carcinoma (HCC) site. Note its hypoechoic pattern as compared with the surrounding cirrhotic liver. This type of tumor is the target of early detection plans because at this stage effective therapy (e.g., resection, transplantation, or percutaneous ablation) may provide long-term disease survival.

Staging of HCC should be based on expert CT or MRI (40). Both techniques confidently detect tumors larger than 2 cm in size, while smaller tumors pose major difficulties in being accurately characterized as benign or malignant. Angiography has currently no role in diagnosis and staging, while lipiodol CT is not reliable (2). Detection of additional tumor sites and vascular invasion

indicates advanced tumor stage. Extrahepatic spread is infrequent at early stages but should be ruled out by chest CT scan. Bone metastases are usually symptomatic and should be evaluated if needed by bone scintigraphy.

Prognostic Prediction

Because cirrhosis underlies HCC in most patients, their prognosis depends on tumor burden, degree of liver function impairment, and the treatment received. At the same time, liver function and tumor extent determines the feasibility of treatment and all parameters should be considered for clinical predictions (41).

Therefore, systems that consider only one dimension such as the tumor-node-metastasis (TNM) classification (42), the Child-Pugh (43), or the Model for End-Stage Liver Disease (MELD) score (44) will be inaccurate. Similarly, scores that just consider general health status and physical capacity such as the performance status (45) or the Karnofsky index will have reduced value. In fact, the sole usefulness of all unidimensional systems is to identify patients with very advanced disease stage and poor short-term outcome. Although there are several scores combining liver function, tumor stage, and/or physical status, most just stratify patients according to expected outcome, but only the Barcelona Clinic Liver Cancer (BCLC) system links staging with treatment indication, and recent studies have validated its usefulness (46,47,48). This was constructed years ago by taking into account the outcome data of several cohort investigations and randomized clinical trials. Patients are divided into the relevant evolutionary stages according to tumor stage, liver function, and presence of symptoms, and within each stratum patients are classified using specific prognostic tools finally linking stage to therapy (41).

Treatment

The only options that can achieve long-term cure are surgical resection, liver transplantation, and percutaneous ablation. They are effective for patients diagnosed at early stages, which represent only less than 40% of the patients. Most cases are diagnosed at a more advanced stage and the only option that has been shown to have a positive impact on survival is transarterial chemoembolization (TACE) (49), but this benefits less than 20% of the advanced cases. Therefore, almost half of the patients will have no option for effective therapy and are candidates for research investigations.

Treatment indication requires a careful evaluation of tumor stage, degree of liver failure, and general health. Figure 44.2 shows the BCLC staging and treatment algorithm. Detection of vascular invasion or extrahepatic spread implies advanced disease stage and precludes any effective therapy. Although there is no functional impairment in patients with a normal underlying liver, in those with chronic liver disease, this aspect is of paramount relevance. Surgical resection is feasible only in patients with well preserved liver function, and decompensated cirrhosis precludes almost all forms of therapy, except liver transplantation.

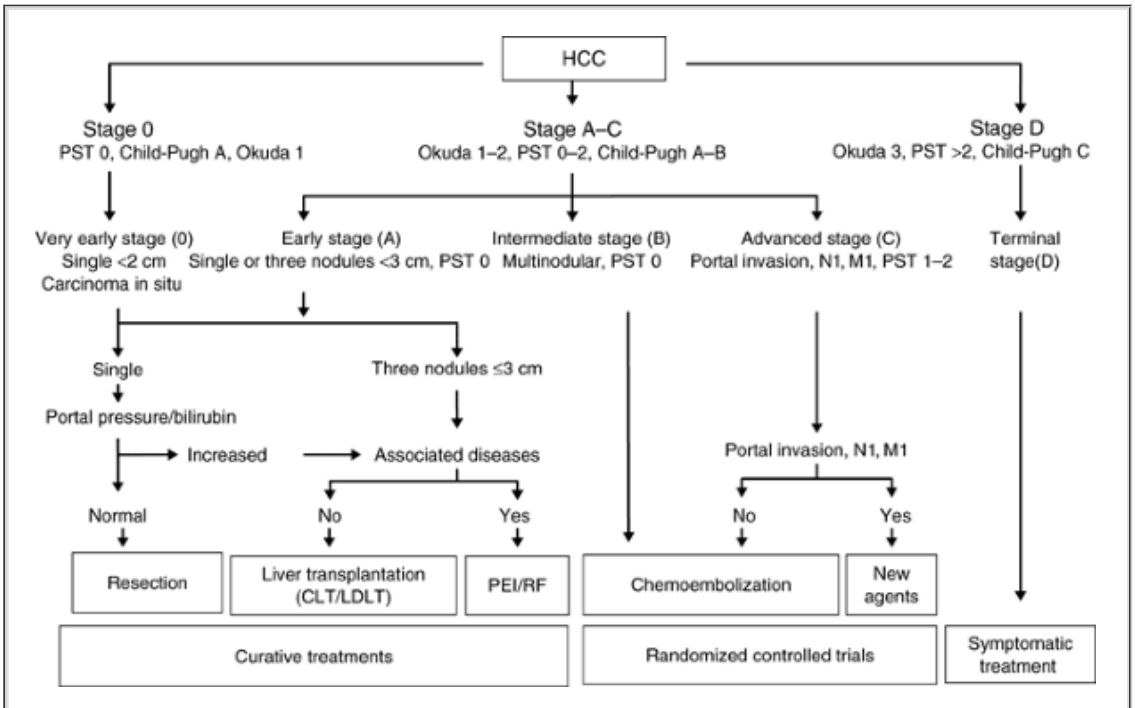
Surgical Treatment

There are no trials comparing resection with liver transplantation and therefore the decision about which should be the first option is highly controversial and should take into account the available resources and the survival expected by applying any of them.

Resection

This is usually restricted to patients with solitary tumors and is the optimal approach for patients with normal liver. Unfortunately, these are less than 5% of the patients with HCC in western countries, and cirrhosis considerably limits surgical resection. Selection of candidates with cirrhosis should aim at a perioperative mortality of less than 3%, a transfusion rate less than 10%, and a 5-year survival rate higher than 50%, and this requires careful selection (50). Allocation into Child-Pugh A stage does not suffice to identify the best candidates for resection. Studies in Barcelona (51) have shown that measurement of portal pressure and bilirubin concentration are the best parameters for this purpose. Patients with normal bilirubin level and without clinically relevant portal hypertension (PHT), defined as a hepatic vein pressure gradient of 10 mm Hg or more, esophageal varices, or splenomegaly with a platelet count of less than 100,000/mm^3, will achieve 5-year survival rates of 70%, whereas this decreases to 50% in patients with PHT and to 25% in those with PHT and a raised bilirubin level. HCC recurrence may affect more than 50% of patients at 3 years and its appearance deteriorates long-term survival (51,52,53). Early recurrence is thought to correspond to tumor spread before resection, while recurrence beyond 2 to 3 years may be due to the emergence of metachronic HCC in a separate cellular clone. The most powerful predictors of postoperative recurrence due to dissemination are

the presence of microvascular invasion, poor differentiation, and satellite lesions. Unfortunately, despite promising reports using interferon, selective radiation, adaptive immunotherapy, and retinoids, there is no effective method to diminish recurrence rate (50).



▪ **Figure 44.2** Barcelona Clinic Liver Cancer (BCLC) staging and treatment algorithm. PST, performance status test; CLT, cadaveric liver transplantation; LDLT, live donor liver transplantation; PEI, percutaneous ethanol injection; RF, radiofrequency ablation. (Modified from Llovet JM,

Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003;362 (9399):1907–1917.)

Transplantation

Transplantation provides excellent outcomes if restricted to patients with early stage disease, as defined by the Milano criteria: Solitary HCC less than 5 cm in size or with up to three nodules, each measuring less than 3 cm in size (54) (Fig. 44.3). Survival of patients selected according to this definition exceeds 70% at 5 years (50). Disease recurrence is approximately 15% and affects mostly the liver, lymph nodes, lung, and bones. Recurrence is more frequent if pathology evidences vascular invasion (macro- or microscopic) or additional tumor nests, characteristics that are highly prevalent in tumors exceeding 5 cm in size (55,56). The main limitation for liver transplantation is the scarcity of livers. As a result, the number of candidates exceeds the number of resources, which implies a waiting time between enlistment and transplantation. During this period the tumor may progress and impede successful therapy (51,57). Priority policy to advance patients with high risk of exclusion because of tumor progression has been attempted but still has to be refined to ensure equity between tumor and nontumor patients and avoid transplantation in those patients with more aggressive disease and therefore worse poor-term outcome. Locoregional treatments such as percutaneous ablation or chemoembolization are considered when

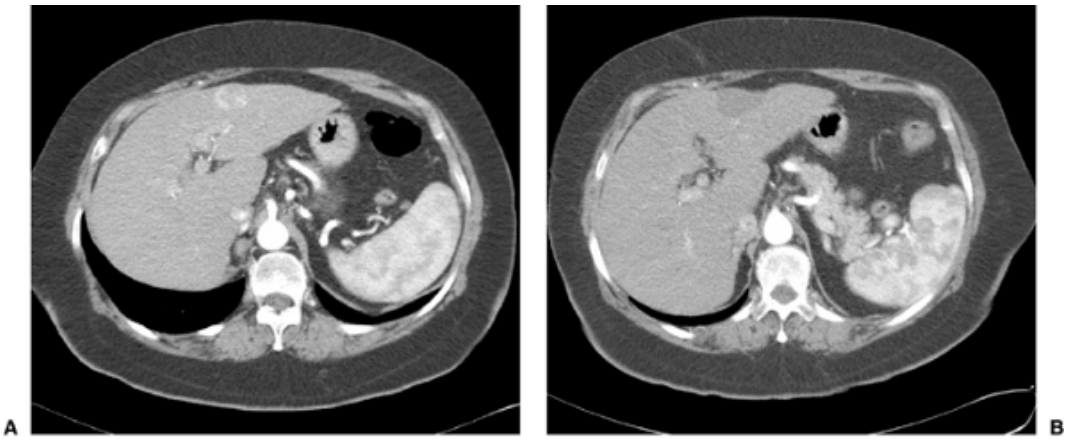
the waiting time exceeds 6 months because these techniques are able to delay tumor progression and hence decrease the risk of exclusion (58,59). The option that has raised more expectancy is live donor liver transplantation. This requires a healthy donor to offer the right or left hepatic lobe that would be implanted into the recipient. Donation implies a 0.5% donor mortality risk, and recent data from Japan suggest that outcome after live donation is very similar to that for cadaveric transplantation (60). At the same time, the validity of the Milano criteria for selection of the optimal candidates has been shown.

▪ **Figure 44.3** Explanted liver showing a hepatocellular carcinoma measuring

3 cm in size within a cirrhotic liver. The tumor has well-defined margins and exhibits a thin pseudocapsule. No satellite nodule is observed and the vessels appear free of invasion at macro- and microscopic examination. This pathology profile indicates a low recurrence risk during follow-up.

Percutaneous Treatments

Percutaneous treatment is a therapeutic option that has rapidly grown during the last decade. Destruction or ablation of tumor cells can be achieved by the injection of chemical substances (e.g., ethanol, acetic acid, and boiling saline) or by the insertion of a probe that modifies local tumor temperature (e.g., radiofrequency ablation, microwave, laser, and cryotherapy). This procedure can be done percutaneously with minimal invasiveness or during laparoscopy and is currently considered the best option for patients with early HCC who are not candidates for surgery. Treatment is repeated on separate days, and its efficacy is evaluated at 1 month by dynamic CT, in which the absence of contrast uptake reflects tumor necrosis (Fig. 44.4). However, some of the tumors initially classified as completely necrosed present as intratumoral recurrence later on; this should be seen as treatment failure that has not been detected immediately after therapy. Recurrence rate after percutaneous ablation is similar to that seen after surgical resection and presents as separate nodules in the vicinity of the earlier ones or in separate liver segments (61).



▪ **Figure 44.4 A:** Dynamic arterial computed tomography (CT) scan shows a tumor with heterogeneous arterial vascularization located in the left lobe corresponding to a solitary hepatocellular carcinoma less than 20 mm in size. **B:** Impaired liver function and associated conditions prevented resection or transplantation and, therefore, the tumor was treated by percutaneous injection of ethanol. Dynamic CT scan evidences that no enhancement is present in the treated tumor, which now appears as a hypodense necrotic residual lesion.

For several years, percutaneous ethanol injection (PEI) under ultrasonographic guidance was the optimal approach. Complete tumor necrosis is achieved in 90%

to 100% of HCCs smaller than 2 cm in diameter, while success is lower in larger tumors. This is due to the presence of septa that prevent diffusion of ethanol, a limitation that does not exist for radiofrequency ablation. This might be performed through single or multiple cooled-tip electrodes, percutaneously, laparoscopically, or intraoperatively. Its efficacy is similar to that of ethanol injection in HCCs less than 2 cm and is superior in larger lesions (62,63,64,65). In addition, the number of treatment sessions is less, and as a whole, radiofrequency ablation has become the preferred approach. However, there are specific locations (e.g., near to main biliary tree, abdominal organs, or heart) where application of radiofrequency is contraindicated because of the risk or severe complications, and this still leaves ethanol as a useful option. Several randomized controlled trials (RCTs) (62,63,64,65) have confirmed this superiority for the local control of tumor, although its impact on survival is less well established. The 3-year survival of patients treated percutaneously exceeds 50% at 3 years, and it has been shown that initial response to treatment is an independent predictor of better survival (61). Therefore, the 5-year survival of Child-Pugh A candidates with a complete response may exceed 50%.

Palliative Therapy

There are many options that have been proposed for patients with HCC, but unfortunately only chemoembolization has been recognized to have a beneficial

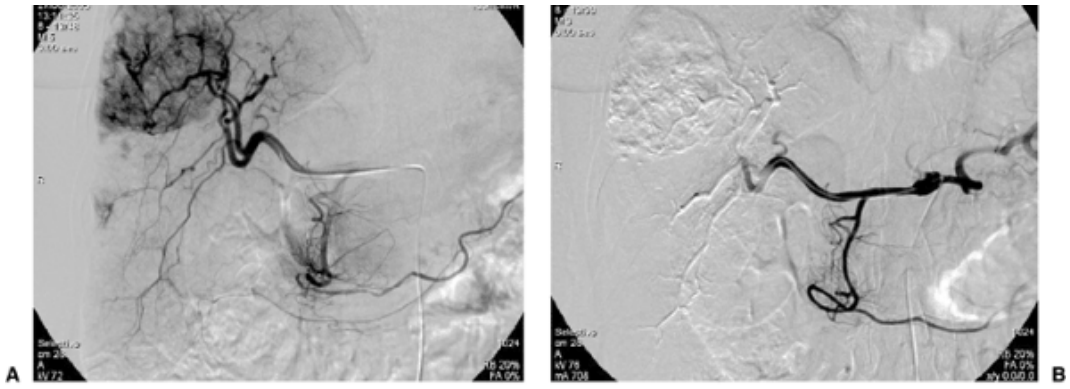
impact on survival (49). Systemic chemotherapy has no effect (<10% objective responses) and is frequently associated with toxicity and hence is usually not recommended. Estrogen blockade with tamoxifen has no benefit even if used at high doses, when it can also become toxic (49). Antiandrogenic therapy is also ineffective and induces hepatic toxicity that might even impair survival (66). Octreotide (67), vitamin D derivatives, and interferon (68) have also been tested with negative results. Radiation therapy has some effect but the need to avoid actinic hepatic damage has prompted the development of strategies for selective tumor targeting using highly focused equipment or intra-arterial injection of lipiodol ^{131}I (69) or radioactive spheres that have shown to have some efficacy, but their impact on survival is still unknown.

Transarterial chemoembolization

Because the blood supply to HCC comes mostly through the hepatic artery, any intervention that blocks this vessel will result in tumor ischemia and necrosis of variable extent. Obviously, the absence of portal blood flow (e.g., portal vein obstruction, portosystemic anastomosis, or hepatofugal flow) is a contraindication for the procedure that is also not indicated in patients with extrahepatic spread. Liver function should be preserved, and this limits its application to patients in Child-Pugh A class.

Hepatic artery obstruction requires an angiographic procedure with advancement of a catheter into the hepatic artery to interrupt blood flow to the tumor as selectively as possible and, therefore, to limit the injury of surrounding nontumor liver. There are several agents that can be used for arterial obstruction. The most common is Gelfoam prepared as 1 mm cubes, but active research aims to develop more effective obstructing agents (70). Mere obstruction of the hepatic artery is known as *transarterial*, or *bland*, *embolization*, whereas when combined with prior injection of a chemotherapeutic agent (e.g., doxorubicin, mitomycin, or cisplatin), the procedure is known as *TACE* (Fig. 44.5). The intervention is well

tolerated. More than half of the patients present with the so-called postembolization syndrome consisting of fever, abdominal pain, and a moderate degree of ileus. This recovers in 48 hours and overlaps with the potential side effects of chemotherapy.



▪ **Figure 44.5 A:** Selective hepatic angiography showing a large hepatocellular carcinoma located in the right lobe. Additional sites were also recognized and, according to staging, the appropriate treatment provided was chemoembolization. **B:** After injection of the chemotherapeutic agent suspended in lipiodol and arterial obstruction with Gelfoam cubes, the tumor vascularization was completely abolished. This combined therapy resulted in extensive tumor necrosis and improved the patient's survival.

Objective responses, as reflected by intratumoral necrosis and reduced tumor burden on dynamic CT scan or MRI, are seen in 15% to 55% of the patients. Objective response is associated with delayed tumor progression and, as a whole, results in an improvement of survival, as shown by recent RCTs (71,72) and cumulative meta-analysis (49).

Future Agents

It should be noted that the current treatment of HCC is mostly directed to the removal of the tumor by physical means. However, tumor development and progression is dictated by biologic events that are currently being actively investigated. As a result, new therapeutic agents will be directed to biologic targets and are expected to be highly selective on the tumor. Gene therapy to correct abnormal gene profile has been awaited for long and is still to show safety and benefit. On the contrary, several new molecules blocking tyrosine kinase signaling, angiogenic activity, or antiapoptotic tumor defense are entering human evaluation and hopefully will result

in a sharp change in the therapeutic management of these patients.

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This article reviews all the options that can be offered to patients with intermediate/advanced hepatocellular cancer*and offers evidence-based data that has been produced during the recent years to define what treatments offer a survival advantage and which ones are of no use or require further investigation*.

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Chapter 45

Surgical Options for the Treatment of Hepatocellular Carcinoma

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Key Concepts

- Hepatocellular carcinoma (HCC) is among the most common fatal solid tumors worldwide. Surgical intervention provides the best hope for cure.
- Most patients with HCC are not candidates for surgery because of severe underlying liver disease, extrahepatic spread, larger tumor size, and major vascular involvement.
- The best surgical results are achieved in patients who have solitary lesions less than 5 cm in diameter or three or fewer multiple tumors, none measuring more than 3 cm. Absence of micro- or macrovascular invasion is also associated with a better long-term prognosis.
- Preoperative ablative measures are commonly performed in patients awaiting liver transplantation for HCC with the hope of improving long-term survival or preventing tumor growth while waiting. There is very little evidence that these treatments are effective for either the prevention of dropout from the waiting list or improvement of long-term outcome.
- Liver transplantation offers the advantage of total hepatectomy, removing not only the tumor but also the diseased liver. However, lifetime immunosuppression carries substantial risks, including enhancement of tumor growth in those patients in whom metastatic disease was not detected before surgery.
- Shortage of donor organs, which has traditionally limited the use of transplantation, has been partially addressed by the use of living donors. However, the risks and benefits for both donor and recipient remain undefined.

As emphasized elsewhere in this text, both the worldwide and North American incidence of chronic hepatitis type C virus (HCV) infection has not yet reached its peak. Only recently have we begun to recognize the potential threat of hepatocellular carcinoma (HCC) that two decades of proliferation of HCV infection may carry. In the continued absence of effective nonsurgical means for either prevention or treatment of HCC secondary to HCV infection, surgery remains the mainstay of our attempts to provide durable cures. Although resection has long been advocated as the first choice for those patients whose general condition, degree of liver dysfunction, and total tumor burden allow it, those patients with underlying chronic liver disease face a lifetime risk of developing additional malignancies in the diseased liver left behind. The appeal of total hepatectomy with transplantation is tempered by the lack of adequate donor livers, the risk of lifetime immunosuppression and what has become a dishearteningly high risk of recurrent hepatitis for those with chronic HCV.

What follows is a brief review of the variety of surgical options available at the beginning of the twenty-first century to treat HCC. Although our chapter in a prior edition of this text serves as the foundation, the current chapter has been completely rewritten with an eye toward updating both the data and the attitudes of

the authors and others toward the components of the varied armamentarium.

Preoperative Assessment

Patients with HCC without distant disease are candidates for surgical resection or other methods of tumor elimination. Standard preoperative work-up should include ultrasonography of the liver, computed tomography (CT) scan of the abdomen, pelvis, and thorax. Suspicious lesions found during metastatic evaluation should be biopsied using percutaneous techniques, if safe and practical. Histologic documentation of extrahepatic disease obviates curative resection and is considered a contraindication to transplantation.

Preoperative Biopsy

The need to establish a preoperative histologic diagnosis of HCC is controversial to some extent. HCC most often develops in patients with risk factors that include chronic hepatites (type C, HCV or type B, HBV) and cirrhosis. In this group of patients, those with CT scan and ultrasound evidence of a discrete solid mass may not need a tissue diagnosis before proceeding to surgery.

The usual method for establishing preoperative histologic diagnosis is by percutaneous needle biopsy or fine-needle aspiration for cytology. Although Caturelli et al. (1) report that cytology is both more accurate and more

sensitive than standard histology, biopsy with an 18- or 20-gauge needle is the more common approach. Seeding of the needle tract with tumor occurs in as many as 5% of cases (2). Isolated case reports of late recurrence of HCC in subcutaneous tissue illustrate the small risk associated with percutaneous needle biopsy (3,4,5,6).

Levy et al. (7) support their argument for not performing percutaneous biopsy preoperatively with a study involving 65 patients, with known risk factors for HCC and a lesion in the liver seen by radiographic evaluation, who were taken to surgery without preoperative histologic diagnosis. Only two of ten patients with lesions less than 3 cm and serum α-fetoprotein (AFP) levels less than 100 ng/mL preoperatively were found not to have HCC.

In reality, most patients are referred for surgical consideration, having already had a biopsy. It is our practice to proceed without biopsy in those patients with HCV, HBV, or cirrhosis. Patients with normal AFP and with no risk factors for development of HCC may require histologic evaluation to justify surgery. In those instances when the surgeon feels resection or ablation is needed regardless of histology, the value of preoperative histology becomes a matter of preference.

Radiographic Evaluation

Although this textbook provides a more thorough discussion of radiologic evaluation of the liver elsewhere, a brief discussion of our surgical perspective is offered.

Ultrasound is a comparatively cost-effective, noninvasive tool that can identify lesions as small as 1 cm. It also reveals information about the nature of the liver parenchyma. The accuracy, thoroughness, and hence, the value of ultrasound to the surgeon are more dependent on the skills of the operator than is the case with many other modalities of evaluation. Although bone and air interfaces may cause interfering reflections, the vasculature and biliary tree of the liver are generally easily visualized.

Doppler imaging is used to assess the direction of blood flow, patency of vessels and location of vessels in relation to the suspected tumor. Newer variants of ultrasound may increase the diagnostic yield of ultrasound. The use of harmonic ultrasound (8,9) and microbubble contrast (10,11) have been investigated with mixed results. The hope is that these adjuvants to traditional ultrasound will increase the sensitivity and specificity of this noninvasive diagnostic tool.

CT has always been a useful technique for imaging the liver. The use of helical CT with IV contrast gives clear cross-sectional images. Proper timing of the image acquisition to capture sequentially both arterial and portal opacification increases the sensitivity of CT for detecting intrahepatic lesions.

CT angiography allows for the definition of the hepatic arterial anatomy using three-dimensional reconstruction without the invasiveness of traditional angiography. CT arterial portography (CTAP) with angiography has been considered to be the most sensitive method of detecting hepatic masses. It requires arterial puncture with injection of contrast dye into the superior mesenteric artery or the celiac axis. At the time of this writing, CTAP is considered the gold standard against which newer imaging modalities are compared. Undoubtedly, advances in imaging techniques will make invasive angiography unnecessary for the evaluation of the liver. At present, all CT imaging techniques that require the use of intravenous contrast carry the risk of allergic anaphylactic reactions and nephrotoxicity.

Magnetic resonance imaging (MRI) is used for hepatic imaging not only to define parenchymal masses, but also to perform noninvasive cholangiography. MR angiography can also be used to define the hepatic arterial supply. Using either double contrast MRI (12) or ferumoxide-enhanced MRI (13,14), several investigators have shown a promising ability to distinguish between tumor and regenerating nodules in the cirrhotic liver. Traditional T1- and T2-weighted

turbo-spin echo MR images have a low sensitivity for detecting small HCC (15), but the sensitivity using ferumoxide-enhanced MRI rivals that of CT.

Positron emission tomography (PET) represents physiologic behavior of the tissue being imaged. The fluorine-labeled 2-deoxy-2 glucose (FDG) PET scan is the most commonly used test. Malignant tumors have a higher rate of glucose metabolism than nonmalignant tissue, therefore this radiolabeled glucose analog will concentrate in neoplastic tissue after intravenous injection and appear as a "hot" spot on the PET image (16,17). Although FDG PET has proven to be a sensitive test for hepatic colorectal metastases, the sensitivity for detection of HCC ranges from 50% to 70%. PET scan is therefore not currently recommended as a routine diagnostic test for the evaluation of suspected HCC (18).

Usually a combination of imaging techniques will give surgeons the most information for preoperative planning. By taking judicious advantage of the technology currently available, a careful surgeon should be able to identify those patients for whom laparotomy is fruitless owing to extrahepatic spread or unresectability of the liver lesion. The addition of intraoperative ultrasound not only aids in the determination of resectability, but can also define the plane of resection to ensure adequate surgical margins. As technology advances, preoperative imaging will be less invasive and carry less risk for the patient.

Prognostic Assessment

Hepatic resection remains the mainstay of curative treatment for HCC, and apart from liver transplantation, provides the only consistent long-term survival. The prognosis of patients with HCC undergoing surgical treatment is dependent on four major factors: (a) Tumor stage and aggressiveness (grade and differentiation); (b) severity of the underlying liver disease; (c) ability of the patient to withstand the treatment; and (d) proposed intervention. On the basis of an assessment of these factors, almost 75% of patients with HCC will be excluded from curative surgery. Patients deemed to have tumors not suitable for curative resection are offered one or more

of the increasing number of available palliative therapies.

Table 45.1. Child-Pugh Score

Criterion	1	2	3
Serum bilirubin (mg/dL)	<2	2–3	>3
Serum albumin (g/dL)	>3.5	3–3.5	<3
Ascites	None	Easily controlled	Poorly controlled
Encephalopathy	None	Minimal	Advanced
INR	<1.7	1.7–2.3	>2.3

5–6 points, Child-Pugh class A; 7–9 points, Child-Pugh class B; 10–15 points, Child-Pugh class C; INR, international normalized ratio.

Postoperative liver failure (0% to 40%) and intrahepatic recurrence (20% to 60%) are the two main reasons for treatment failure after liver resection (19,20). Prognostic risk factors for intrahepatic recurrence after resection for HCC have been extensively studied and the conventional pathologic features of the tumor are the most widely cited. Large tumor size (>5 cm) (21), gross or microscopic tumor involvement at the resection margin, vascular invasion (22,23), and macro- or microscopic satellite lesions (20,24) all predict a worse outcome after hepatic resection.

By contrast, the risk of postoperative liver failure has been less easy to predict. Although numerous tests and formulas have been proposed, there is no uniformly accepted method to assess functional hepatic reserve. Patients with HCC most often have cirrhosis, and it therefore comes as no surprise that liver failure after resection is a major cause of mortality. There are a number of clinical stratification scores that have been used to predict postoperative liver failure. Of these, the Child-Pugh score is the best established. It provides a score using simple clinical variables (encephalopathy and ascites) and blood tests (serum bilirubin and albumin and prothrombin time) (Table 45.1). Although the Child-Pugh score was originally developed to predict the survival of patients with cirrhosis and variceal hemorrhage undergoing portocaval shunts, numerous studies have failed to confirm its value in predicting morbidity and mortality after liver resection (25). In fact, Bruix et al. have shown that the presence of portal hypertension, defined as a wedged hepatic vein to hepatic vein pressure gradient, greater than 10 mm Hg is associated with hepatic decompensation in up to 60% of patients with Child A cirrhosis after liver resection.

The Okuda staging system combines the assessment of tumor volume and functional hepatic impairment. The variables evaluated with this system are tumor volume, presence of ascites, and serum concentrations of bilirubin and albumin (Table 45.2). The stage of the disease is based on the number of positive values: Stage I = no positives; Stage II = 1 or 2 positives; Stage III = 3 or 4 positives. In the absence of any treatment, median survival for patients with Okuda Stage I, II, and III disease are 8, 2, and 0.7 months respectively (26). Unfortunately, the Okuda system only provides a rough estimation of outcome after surgery. The same is true for

the tumor-node-metastasis (TNM) classification, which does not account for underlying liver dysfunction and in reality, provides little help in the preoperative assessment and decision making for patients with HCC (27).

Table 45.2. Okuda Staging Variables

Staging criteria	Positive	Negative
Tumor size (% of liver volume)	>50%	<50%
Presence of ascites	Detectable	Absent
Serum albumin	<3 g/dL	>3 g/dL
Serum bilirubin	>3 mg/dL	<3 mg/dL

More recently, the Cancer of the Liver Italian Program (CLIP) has proposed a new scoring system, the CLIP score, which includes assessment of both tumor characteristics and hepatic functional reserve. (28,29). The CLIP score

consists of four variables: Child-Pugh Score, tumor morphology, serum AFP level and portal vein invasion. This system overcomes some of the problems encountered with other classifications and may prove to have greater prognostic value.

In an attempt to overcome the limitations of clinical scoring systems, alternative methods of quantifying hepatic reserve such as the bromsulfophthalein retention test, the 14C-aminopyrine breath test, the indocyanine green (ICG) clearance test, and the monoethylglycinexylidide (MEGX) test, which measures the liver's ability to clear a lidocaine metabolite, have been evaluated. The ICG retention test is the most widely used adjunctive test. ICG is actively and solely removed from plasma by the liver. Clearance of ICG is considered to be impaired when 15% or more of the dye remains within the plasma 15 minutes after intravenous injection. These tests have not gained widespread acceptance, mostly because of the cumbersome nature of some of the tests, requiring numerous samples, and the fact that these tests measure only the severity of the underlying liver disease prior to resection and do not give a precise indication of the liver function that will remain following partial hepatectomy. It appears that no single test offers a better prediction of postoperative liver failure than the Child-Pugh score and an experienced surgeon (30).

Liver Resection

Preoperative Evaluation

Hepatic resection inflicts significant physiologic stress on the patient. Patients should be evaluated for possible cardiac disease with screening electrocardiogram (ECG) and a careful evaluation of the patient's level of activity. Hepatic resection carries a high risk for cardiac events according to the classifications presented by Eagle et al. (31). Further cardiac evaluations (stress testing and cardiac catheterization) are usually required for adequate evaluation of patients with risk factors for coronary artery disease. These risk factors include the following:

- Previous history of myocardial infarction
- History of congestive heart failure or angina pectoris
- Diabetes mellitus
- Advanced age
- Abnormal ECG
- Nonsinus rhythm on ECG
- Low functional capacity

Patients with unstable coronary syndromes, decompensated congestive heart failure, significant arrhythmias, or severe valvular disease most often require cardiac catheterization with or without revascularization prior to hepatic resection.

Patients with a known history of pulmonary disease should be evaluated with preoperative pulmonary function tests and an arterial blood gas. Strict exclusion criteria for patients with pulmonary disease are not well established. As with other patients undergoing major abdominal surgery, early ambulation and the use of incentive spirometry may help prevent atelectasis.

Patients undergoing hepatic resection for malignant tumors meet high-risk criteria for deep venous thrombosis (DVT) (32). Recommendations for prophylaxis include intermittent pneumatic compression (IPC) stockings and low-molecular-weight heparin. To reduce the risk of postoperative bleeding, anticoagulants are avoided.

All patients undergoing a hepatic resection should have blood products available for the operating room. We do not routinely use autologous shed blood recovery techniques when resecting malignancies in the liver. Patients with liver disease and prolonged prothrombin times should receive fresh frozen plasma (FFP) to correct the coagulopathy early in the surgery. Midorikawa et al. advocate the use of FFP routinely, both in the operating room and postoperatively, in all hepatic resections (33).

Surgical Resection

Surgical resection and liver transplantation are the only therapies that can be applied with curative intent. Resection is the treatment of choice for noncirrhotic patients with small tumors (<5 cm) and selected cirrhotic patients with minimal decompensation (Child A patients). The data from Barcelona have been quite convincing that if there is any evidence of portal hypertension, particularly hepatocellular dysfunction, the outcome of liver resection is poor. Improved patient selection in conjunction with advances in perioperative

management has resulted in lower operative morbidity and mortality. In general, a five-year survival rate of more than 50% can be expected in well-selected patients (Table 45.3A,B). Unfortunately, long-term survival is negatively affected by tumor recurrence rates over 50%. Recurrence may occur in the liver or at extrahepatic sites. Recurrence occurs not only because of the pathologic aspects of the tumor, but also because the remnant liver still has premalignant potential, with HCV-positive patients being at particular risk (34).

Table 45.3A. Results of Liver Resection for Hepatocellular Carcinoma

Author	Year	Number of patients	30-D operative mortality (%)	Recurrence (%)	Survival (%)			Comments
					1 Y	3 Y	5 Y	
Iwatsuki	1991	76	—	50 (5 y)	71	—	33	17/76 cirrhosis
Ringe	1991	131	8.9–11.2	32	67	42	35	30% cirrhosis
Franco	1990	72	7	22	68	51	—	All cirrhosis
Bismuth	1993	60	10	73	80	52	—	100% cirrhosis
LCSG of Japan	1990	2,174	0	0	67	39.6	28.5	74% cirrhosis; 77% Child A
Takenaka	1996	280	2	17–38 (5 y)	88	70	50	52% cirrhosis
Vauthey	1995	106	6	NR	—	—	41	33% cirrhosis; 95% Child A
Nonani	1997	262	2.7	52.3	67–96	—	17–83	82% cirrhosis
Majno	1997	76	NR	57–81	84–78	57–47	43–35	100% cirrhosis randomized trial of chemoembolization

LCSG, Liver Cancer Study Group; NR, not reported.

Table 45.3B. Results of Liver Resection for Hepatocellular Carcinoma

	Year	N	% cirrhotic	Operative mortality (%)	Operative morbidity	Overall 3-y survival (%)	Overall 5-y survival (%)	3-y tumor-free survival (%)
Gouillat	1999	37	100	11	—	35	24	23
Figueras	2000	35	100	—	—	58	48	44
Hanazaki	2000	386	52	4.1	24.5%	51.1	34.4	36.7
Usatoff	2001	19	47	5	37%	33	11	—
Grazi	2001	264	100	4.9	—	63.1	41.1	49.3
Kanematsu	2002	303	55	3.3	—	67	51	41

The type of resection performed is dictated by the size and location of the tumor. Resections are usually segmental (based on Couinaud segmental anatomy—see Fig. 45.1) or lobar. The specifics of the technical approach to the resection are surgeon-dependent. Most commonly, a bilateral subcostal incision is used. Thoracoabdominal incision plays little or no role in our experience. Initial exploration of the abdomen should rule out extrahepatic disease. The arterial anatomy of the liver should be assessed to identify aberrant left or replaced

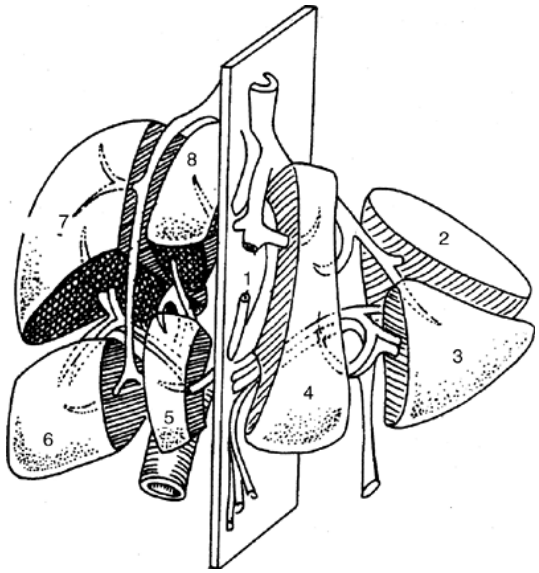
right hepatic arteries.

After the liver is mobilized, resectability of the tumor should be confirmed. The use of intraoperative ultrasound may give the surgeon information about the proximity of the tumor to hepatic veins and portal vessels. It also provides further assurance that no previously unidentified lesions are present in the liver.

Traditionally, the hilar structures (hepatic artery, portal vein and bile duct) leading to the lobe to be resected are identified and individually ligated prior to the parenchymal dissection. A Pringle maneuver may be used to occlude the inflow during the parenchymal division to minimize blood loss. Inflow can be occluded for up to 60 minutes—continuously or intermittently. An alternative approach is total vascular exclusion where the vena cava is isolated above and below the liver and a soft vascular clamp is placed across the hilum of the liver prior to parenchymal division. This technique is useful for segmental and lobar resections. Patients who do not tolerate the lack of venous return may be supported with veno–veno bypass.

Published results of hepatic resection for treatment of HCC in large series of patients underscore the current safety of liver resection in patients with cirrhosis (29,35,36,37,38,39,40,41). Strategies for improved survival include the use of perioperative nutritional support, FFP during and after resection and careful selection of patients. The use of preoperative portal vein embolization to enhance hypertrophy of the uninvolved segment or lobe of liver

can also help decrease the risk of postoperative hepatic failure (42).



▪ **Figure 45.1** Couinaud's segmental hepatic anatomy. (From Couinaud C. Le foie. The numbers refer to the names of each segment. *Etudes anatomiques et chirugicales*. Paris: Masson; 1957, with permission.)

The recurrence rate after resection remains high. Some groups advocate segmental or lobar resection rather than excision of the tumor to decrease the rate of local recurrence (30). Tumor-free margins of resection, lack of vascular invasion, and size of the tumor are variables that help predict the risk of recurrence (Table 45.3A,B).

Ablative Techniques

Most patients with HCC are not candidates for hepatic resection. This is because of multifocal intrahepatic disease, extrahepatic tumor, inadequate functional hepatic reserve, or involvement of the portal vein bifurcation. (43) For these reasons, several techniques have been developed in order to provide therapeutic options for this subgroup of patients with HCC. Treatment modalities include systemic and regional chemotherapy, percutaneous ethanol or acetic acid injection, and cryotherapy and thermal techniques such as laser, microwave, and radiofrequency ablation (RFA) (38,44). Here we describe the current surgically applicable ablative techniques, cryotherapy and RFA.

Cryotherapy

Hepatic cryotherapy employs the delivery of subfreezing temperatures by the placement of a cryoprobe directly into the tumor. At present, this technique requires a laparotomy or laparoscopy as well as sophisticated equipment that use liquid nitrogen or argon as the freezing agent. (45,46,47) Cell death is the consequence of intra- and extracellular ice crystal formation with the resultant destruction of cellular structures. (42,48,49) An ice ball is created around the tip of the probe. The development of the ice ball is monitored intraoperatively by ultrasound. A freeze–thaw cycle is usually repeated to achieve adequate tumor destruction; the freeze cycle is 10 minutes long followed by 5 minutes of thawing. This sequence is repeated on each lesion. The probe is then gently removed, the tract packed with Gelfoam, and manual pressure applied to control bleeding. Larger lesions require

the placement of multiple probes to achieve the required ablation margin of 1 cm of hepatic parenchyma around the tumor.

Complication rates range from 10% to 50% and mortality from 0% to 4%. Complications include hypothermia and its associated coagulopathy, bleeding, biliary fistula, liver surface cracking, and intrahepatic and subphrenic abscess. A rare complication is "cryogenic shock syndrome," a form of multiorgan failure and disseminated intravascular coagulation (38,40,50). The results of most clinical studies of cryotherapy for hepatic malignancies are difficult to interpret. Nonetheless, a study by Zhou et al. demonstrated acceptable long-term survival in 235 patients with HCC, although most patients in the study received some form of therapeutic intervention in addition to cryotherapy (51). Local early tumor recurrence rates are reported to be approximately 15%. The high risk of recurrence and the high complication rate have contributed to making cryotherapy a fairly unpopular mode of treatment.

Radiofrequency Ablation

The concept of thermal ablation of hepatic tumors is not a novel one. However, not until recent technologic advances made it possible to induce a predictable zone of tissue destruction did it become a practical tool. RFA of hepatic tumors has been the focus of increasing research recently. The technique involves delivery of high-frequency alternating current from a needle electrode to the tissue; the parenchyma is subsequently heated to above a cytotoxic temperature (42°C). The higher the temperature generated in the tissue the lower the exposure time required to cause cell death (38,43,52).

The simple straight needle electrodes are no longer used; modern RFA needles have the so-called multiple array hook electrodes that retract into the needle shaft. The needle shaft is an insulated 14- to 18-gauge needle of varying lengths (usually 15 to 25 cm). As

with cryotherapy, it is usual to perform RFA through laparotomy or laparoscopy. The needle is placed into the tumor under ultrasound guidance. The multiple array hooks are then deployed into the tumor. Once deployed, the array creates an umbrella-like cluster of electrodes with a 3.5 or 5.0 cm diameter. These electrodes can produce a zone of coagulation necrosis up to 4 to 7 cm in diameter.

Most series of RFA for HCC report a relatively low complication rate in the region of 2% to 3%. Careful surgical technique and good preoperative planning allow the avoidance of thermal injury to adjacent structures such as diaphragm and bowel. Early recurrence occurs in 2% to 5% of patients, with recurrences occurring at the periphery of the lesion, most likely related to incomplete ablation at the time of surgery. The recurrence rate with RFA is notably lower than that associated with cryotherapy. The study by Curley et al. demonstrated acceptable results, with 60% of their patients alive without radiographically detectable tumor recurrence at a median follow-up of 19 months (53). In a more recent review of their data, they show that 55.4% of their patients were alive at 5 years after RFA for tumors averaging 3.3 cm in diameter (53).

At present, RFA offers a relatively safe and effective treatment for patients with HCC who are not candidates for liver resection or liver transplantation. In comparison to other therapies, RFA and percutaneous ethanol injection (PEI) appear roughly comparable for smaller tumors. However, for tumors larger than 3.5 cm, the same ablation usually requires multiple outpatient PEI sessions, compared to a single inpatient treatment session with RFA. We currently recommend RFA over PEI and cryotherapy for unresectable HCC. PEI is reserved for small, unresectable HCC, such as for local control of small tumors in patients awaiting liver transplantation.

A newer therapy on the horizon is hepatic arterial infusion of Yttrium-90 (TheraSphere), which has been reviewed by a group in Pittsburgh. Sixty-five patients with unresectable, biopsy-proven HCC were treated. Twenty-five of the 64 patients had a partial response to therapy, as shown by CT scan. Deaths following the treatment were attributed to liver failure, HCC progression, and metastatic disease. There are no comparisons to a similar cohort to determine whether the TheraSpheres played a role in the demise of these patients (54). Another group has reported partial response rates of 78% (55) and there have been small single-center safety studies that conclude that patients with intact hepatocellular function can tolerate this therapy (56,57). This is a limited experience to date and large studies of safety and efficacy are needed before it is accepted as standard therapy.

Liver Transplantation

The first patient to survive liver transplantation for more than a year was a child with HCC. Foreshadowing the failure of our attempts to cure liver cancer reliably with total hepatectomy, the patient died with recurrent tumor 400 days following transplantation. Our collected experience during more than three decades since represents a frustrating mix of miraculous success and tragic failure. Nonetheless, total hepatectomy with transplantation remains the most attractive alternative for treating HCC, not only because it removes the diseased liver that is often associated with HCC, but also because it provides the widest surgical margins with low risk for hepatic failure.

On the basis of a review of the data available till 1983, a National Institutes of Health consensus development conference that year endorsed the use of liver transplantation for treatment of selected patients with HCC. As liver transplant centers proliferated throughout North America and Europe, wider experience underscored a serious concern. Although providing a chance for cure to patients with no other alternatives, when compared to the results for transplantation in the treatment of other liver diseases, the long-term survival probability for patients who underwent transplantation for HCC was disappointing in most and unacceptable in others. Five-year survival rates reported between 1985 and 1989 from three leading transplant centers were 25% to 40% (58,59,60). In 1991, Penn, relying on data submitted by numerous transplant centers to the Transplant Tumor Registry, reported that the overall 5-year survival observed was 18% (53). These reports arrived at a time when the shortage of donor livers was beginning to drive waiting times for most patients beyond the 1-year mark, leading to an

alarming increase in the risk of death for waiting candidates. With 5-year survival probabilities for other types of liver transplant recipients reportedly in excess of 80% to 85%, advocates for providing liver transplantation to patients with liver cancer were obliged to improve their results.

In the mid-1980s, investigators began focusing on two major strategies for improving results. One approach involved better patient selection through examination of retrospective information about tumor staging and histologic grade. The other included a variety of pre- and post-transplant treatment regimens designed to lessen the risk of recurrence.

Early reports suggested and subsequent experience has confirmed that the most favorable results are obtained in patients with so-called incidental HCC. These are tumors that were not known to be present prior to liver transplantation but that were discovered during careful examination of the resected liver. In the experience reported by Iwatsuki et al. in 1991,

no patients in this category experienced recurrence of tumor during the follow-up period (61). The same report, as well as data from other authors, has confirmed that among patients with known HCC, those with small tumors fare better than those with larger ones. Remarkably, in 1996 Mazzaferro et al. from Milan reported that 85% of patients with a single tumor less than 5 cm in diameter and 92% of those with three or fewer tumors, none larger than 3 cm, experienced a 4-year tumor-free survival (62).

Some authorities have advocated the TNM staging system as the standard method for comparing the results of various studies and for selecting patients for treatment with orthotopic liver transplantation (Table 45.4). Three previous studies have shown that patients with TNM stages I and II disease, particularly those with severe underlying chronic liver disease, obtain a survival benefit from transplantation compared to either resection or nonsurgical options (53,63,64). However, patients with stage III C and IV disease have a poor prognosis with all treatment options, including transplantation. At the same time, the authors and others have previously emphasized that patients with stage III disease are a heterogeneous group, particularly because patients with large (T3) tumors but without nodal disease are considered stage III. The presence or absence of nodal disease among stage III patients is thought to explain the striking difference between a 57% and a 16% five-year survival reported for patients undergoing transplantation in two large series in 1991 (53,55).

Factors with a demonstrated association with a poor prognosis for patients undergoing liver transplantation for HCC include male gender, tumor size, multiplicity, bilobar involvement, lymph node metastases, cirrhosis, and extrahepatic tumor (49,54,55,65,66,67,68). Histologic evidence of microvascular invasion has also been associated with an increased risk of tumor recurrence. All too often, in practice, the more important histologic factors are only determined on careful examination of the explanted native liver days after the transplantation.

The prognosis for allograft recipients treated for cancer is further affected by the need for long-term immunosuppression. In 1991, researchers at Pittsburgh examined the acceleration of growth rates among patients on antirejection medications with recurrent HCC (69). Despite continued advances in immunosuppressants in the subsequent decade, both cyclosporine and tacrolimus remain mainstays in modern maintenance protocols. Because both are potent inhibitors of interleukin-2 (IL-2) production, the mechanisms of natural tumor surveillance requiring natural killer cells and lymphokine-activated killer cells may be markedly reduced among transplant recipients (70,71).

Table 45.4. Staging of Hepatocellular Carcinoma

Stage	Primary tumor	Nodal status	Distant metastases
I	T1	N0	M0
II	T2	N0	M0
III	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
IVA	T4	Any N	M0
IVB	Any T	Any N	M1

Using known risk factors for recurrence of HCC, the Pittsburgh group has attempted to predict both the risk of tumor recurrence and the time to recurrence of HCC after liver transplantation. In 1996, Marsh et al. examined five known risk factors (gender, tumor number, lobar distribution, tumor size, and presence of vascular invasion) in an effort to assign patients to one of three groups:

1. Patients who should not develop recurrent HCC and therefore do not require adjunctive chemotherapy.
2. Patients who will suffer recurrence and for whom adjunctive chemotherapy increases survival time.
3. Patients who may or may not develop recurrent HCC and for whom recurrence may be prevented by chemotherapy.

The authors believed that by categorizing patients with their scoring system, they could use adjuvant treatment only for those patients who are most likely to benefit. The study was an exercise in statistics, however, and the model had yet to be validated with clinical data (72).

More recently, Iwatsuki et al. from Pittsburgh have further challenged the conventional wisdom that TNM staging offers useful prognostic information (73). Retrospective analysis of 344 consecutive patients who underwent liver transplantation for treatment of nonfibrolamellar HCC led to the development of a Prognostic Risk Score (PRS). Multivariate analysis showed that bilobar distribution of the tumor, tumor size of 2 to 5 cm or greater than 5 cm, and microscopic or unaided visual evidence of vascular invasion were the three most important predictive variables. PRSs of patients could be used to assign them to one of five categories of risk for recurrence (Table 45.5).

In 1983, Koo et al. recovered malignant tumor cells from the right atrium of patients undergoing hepatectomy for liver cancer (74). We first observed in 1985 that the most common pattern of recurrence for HCC following liver transplantation was a multifocal spread in the newly transplanted liver. We speculated then that efforts directed at circulating tumor cells might effectively reduce the risk of recurrence. A decade later, Kar and Carr showed that patients with HCC can have as many as one billion tumor cells in the

systemic circulation each day (75). Therefore, the use of neoadjuvant chemotherapy or pretransplant arterial chemoembolization has received widespread attention during the last decade (Table 45.6).

Table 45.5. Prognostic Risk Score

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
PRS	0 to <7.5	7.5 to 11.0	>11.0 to 15.0	>15.0	+ nodes or DM
5-y tumor-free survival	100%	61%	40%	5%	0%

Prognostic Risk Score is calculated on the basis of bilobar distribution, tumor size 2–5 cm or >5 cm and vascular invasion. PRS, prognostic risk score; DM, distance metastases.
From Iwatsuki S, Dvorchik I, Marsh JW, et al. Liver transplantation for hepatocellular carcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 2000;191(4):389–394.

The theoretical advantages of pretransplant chemoembolization are attractive. Because most, if not all, of the blood supplied to HCC is from the arterial system, the ischemia induced by arterial embolization makes the tumor more susceptible to the effects of the applied chemotherapeutic agent. In theory, one can also deliver higher doses of chemotherapeutic agents directly to the tumor than that possible through systemic use.

We and others have used chemoembolization both in an elective setting at the time the tumor is first discovered, and at the time of liver transplantation. Intraoperative chemoembolization is intended to reduce the number of viable tumor cells that liver mobilization associated with total hepatectomy releases into the bloodstream. For patients on the waiting list for transplantation, chemoembolization has been used to reduce the risk of continued tumor growth as well as to reduce the risk of spread at the time of hepatectomy.

In nonrandomized applications, several authors believe they have obtained improved survival with both methods of chemoembolization. In 1997 Majno et al. from Paris reported the successful use of chemoembolization to downstage tumors that were more than 3 cm in diameter (76). In some patients, they observed complete necrosis of the tumor. Patients with these responses who underwent subsequent transplantation experienced a significant improvement in tumor-free survival compared to patients with either an incomplete response or to those who did not receive chemoembolization (Fig. 45.2). More recent studies, though, have not shown a significant survival advantage to those patients undergoing transarterial chemoembolization (TACE) and liver transplantation (77,78). Tumor size seemed to be one of the most important prognostic factors among the patients in these reports, with those having a tumor greater than 5 cm in diameter experiencing a ten times greater incidence of recurrence than those with smaller tumors.

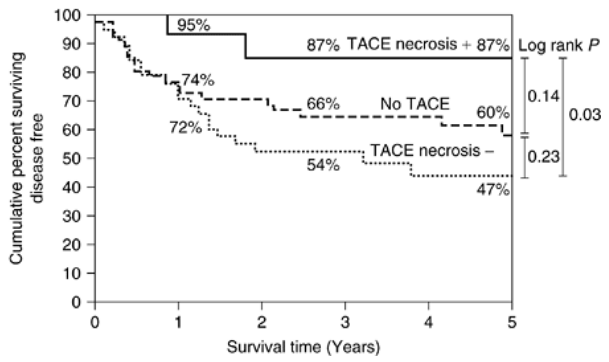
Table 45.6. Adjuvant Therapy with Liver Transplantation for Hepatocellular Carcinoma

Author	Year	N	Therapy	Survival	Recurrence (%)

Bismuth	1992	20	Arterial chemoembolization with ethiodized oil	2 y (49%)	—
Carr	1993	11	Pretransplant interferon arterial chemoembolization with doxorubricin and cisplatin postoperative chemotherapy	1 y (91%)	—
Stone	1993	20	Pre- and postoperative doxorubricin intravenously	3 y (59%)	45
Cherqui	1994	9	Preoperative chemoembolization and radiation (5 Gy); postoperative mitoxantrone	3 y (64%)	33
Olthoff	1994	25	Postoperative chemotherapy with doxorubricin and cisplatin fluorouracil	3 y (46%)	20
Majno	1997	54	Chemoembolization with lipiodol mixed with doxorubricin or cisplatin	5 y (55%)	—

As the waiting time for liver transplant candidates with hepatic tumors continued to increase during the last half of the 1990s, the effectiveness of preparative regimens for tumor control waned dramatically. The United Network for Organ Sharing (UNOS) allocation algorithm for cadaveric-donor livers assigned priority to patients with liver dysfunction. Patients with HCC but with preserved hepatic function were not able to

receive adequate priority until their waiting time (also a factor in the allocation algorithm) exceeded 1 to 2 years, depending on geographic location. Inevitably, continued tumor growth during the prolonged waiting period often obviated successful transplantation either because candidates developed evidence of metastatic disease and were removed from the waiting list or because those that finally received a graft were more likely to have developed unrecognized metastases by the time they received the transplant.



▪ **Figure 45.2** Cumulative disease-free survival after liver transplantation for hepatocellular carcinoma associated with cirrhosis: Complete necrosis of the lesion after transarterial chemoembolization TACE (necrosis positive: *n* = 15), no necrosis after TACE (necrosis negative: *n* = 39), and no TACE (*n* = 57). (From Majno PE, Adam R, Bismuth H, et al. Influence of preoperative transarterial lipiodol chemoembolization on resection and transplantation for hepatocellular carcinoma in patients with cirrhosis. *Ann Surg* 1997;226 (6):688–703 with permission.)

Although UNOS eventually allowed special consideration to patients with favorable hepatic tumors by allowing them to compete in a higher status category normally reserved for patients with severe liver dysfunction, the leaders of many liver transplant programs began advocating additional measures to provide expedited liver transplantation. In addition to the general advocacy to increase the available supply of cadaveric-donor livers through wider use of split-livers (79,80,81), many programs began examining the risks and benefits of using live volunteers to provide donor livers. Using a statistical decision analysis technique that considered a cohort of hypothetical patients with compensated Child A cirrhosis and an unresectable 3.5 cm HCC, Cheng et al. from Boston satisfied themselves that live-donor adult-to-adult liver transplantation offered a 4.5-year increase in tumor-free survival as compared to waiting for cadaveric-donor liver transplantation or no transplant. The advantage persisted in their model even in the face of varying severity of cirrhosis, age, tumor doubling time, tumor growth pattern, blood type, regional transplant volume, initial tumor size, and rate of progression of cirrhosis (82). A similar experience was reported by the Mount Sinai Hospital during the period from 1998 to

2001—the average waiting time for a deceased donor was 414 days as compared with 83 days for a living donor—proposing that HCC is an ideal indication for living-donor liver transplantation (83).

In February 2002, the Model of End-Stage Liver Disease (MELD) system was implemented in the United States. Livers from cadaveric donors are now allocated according to a patient's score that is based on their international normalized ratio (INR), creatinine level, and bilirubin level. Patients with HCC most often do not have decompensation of their cirrhosis so the transplant community decided to implement a separate point system that would provide HCC patients with access to organs before their disease progressed beyond the point that they were eligible for transplantation. The Milan criteria have been used to define which patients are eligible for liver transplantation by UNOS standards, that is, patients must have one tumor less than 5 cm or up to three tumors, none of which may exceed 3 cm in diameter. Documentation of the presence of tumor is through imaging and a positive biopsy, AFP more than 200 ng/mL, or previous ablative therapy of the lesion. Points were assigned on the basis of prediction of survival for patients with stage I and II disease (84). With the introduction of the MELD system for liver allocation in adult patients who underwent transplant, the incidence of transplantation for HCC has risen dramatically. In 2001, 2.8% of liver transplants in the United States were for a diagnosis of HCC with or without cirrhosis, and this rose to 7% (434 of 6,186) in 2004. Since the origination of the MELD system, the point allocation for patients with HCC has been adjusted and currently only patients with Stage II disease are eligible for point upgrades. Patient survival rates after liver transplantation for HCC are acceptable, with the 5-year survival being 61.1% in a recent review

of patients transplanted between 1996 and 2001. This rate is lower than that for patients transplanted without HCC (5-year survival approximately 70%), but remains acceptable (85).

As the full impact of our worldwide epidemic of HCV infection reaches its acme, the prospect of a similarly overwhelming population of patients with HCC ought to cause us considerable alarm. Whereas HCC may best be prevented by more effective early treatment of viral hepatitis, the absolute number of patients with established cirrhosis that are at risk for cancer—although a relatively small percentage of the total—is likely to be staggering. Therefore, under the assumption that transplantation offers the best chance of tumor-free survival for these patients, and with the supply of donor livers from cadaveric sources falling even further behind demand from all patients with liver disease (many with far better long-term survival prospects than patients with tumor), the burden on families, friends, and other volunteers to provide pieces of their livers should give us all pause. Although a case-by-case focus on the needs of our own patients ought to be our primary role as physicians, a more global oversight of our results, with a particular emphasis on donor safety and health, needs to be established.

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Chapter 46

The Liver in Pregnancy

Yannick Bacq

Key Concepts

- During normal pregnancy, except for alkaline phosphatase, most values of serum liver tests remain below the upper normal limits established in nonpregnant women. Consequently, increased levels of aminotransferases, bilirubin, or serum bile acids usually indicate the presence of liver disease. By contrast, an increase in serum alkaline phosphatase levels in pregnancy is not specific for liver disease because it may be of placental origin.
- The liver disorders that occur in pregnancy can be divided into three groups: (a) Liver diseases unique to pregnancies that are specifically pregnancy related; (b) intercurrent liver diseases in pregnancy, that is, acute liver disease occurring fortuitously during pregnancy; and (c) chronic liver diseases that may be revealed by pregnancy, or more often diagnosed fortuitously during pregnancy.
- It is essential that liver disease in pregnancy is recognized and understood because certain disorders can threaten the lives of both mother and infant.
- Liver disorders unique to pregnancy include the exceptional primary hepatic pregnancy, liver dysfunction associated with hyperemesis gravidarum, intrahepatic cholestasis of pregnancy (ICP), liver disorders of preeclampsia including hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, and acute fatty liver of pregnancy (AFLP).
- ICP is a benign disease for the mother but carries a risk for the baby because of the possibility of premature delivery and sudden fetal death. Generalized pruritus is the main symptom. Serum bile acid and aminotransferase levels are increased, although the γ-glutamyl transpeptidase levels may be normal or only slightly increased.
- AFLP is a form of hepatic failure associated with coagulopathy and occasionally encephalopathy and hypoglycemia. An association has been found between AFLP and a defect of long-chain 3-hydroxyacyl coenzyme A dehydrogenase (LCHAD) in the fetus. Women in whom AFLP develops and their offspring should undergo deoxyribonucleic acid (DNA) testing for the main associated genetic mutation (G1528C) in the gene coding for LCHAD.
- Certain liver diseases that can occur in anyone, pregnant or not, are more severe during pregnancy (e.g., viral hepatitis E and herpes simplex hepatitis). Other disorders can be precipitated by pregnancy or during postpartum, such as cholelithiasis and Budd-Chiari syndrome.
- Pregnancy in patients with advanced chronic liver disease is rare, although

patients with treatable liver diseases, such as autoimmune hepatitis and Wilson disease, may regain fertility and should be maintained on treatment during gestation. By contrast, successful pregnancy in patients with mild chronic liver disease, such as viral hepatitis B or C, is common. Pregnancy after liver transplantation may be associated with prematurity and increased maternal complications, but not with teratogenicity.

Liver disease in a pregnant woman can make the consulting physician uneasy. Most gastroenterologists and hepatologists are unfamiliar with the pregnant state and the liver diseases associated with it, and pregnancy is rarely seen in patients with severe chronic liver disease. Also, the stakes are higher for these patients, given the presence of a second life in the form of the fetus. The distress on the part of the consultant is well founded because pregnancy is a state of altered, although normal, physiology. Certain disorders of the liver are unique to pregnancy and not comparable with liver diseases in nonpregnant patients. Furthermore, some conditions that can affect anyone, pregnant or not, may follow an unusually severe course in the pregnant woman. Despite these problems, the care of these otherwise young and healthy women is gratifying, and most return to good health simply by being delivered of the infant. Recent advances in our understanding of liver diseases during pregnancy have simplified and rationalized the approach to these patients.

The Liver in Normal Pregnancy

The changes involving the liver during normal pregnancy have been discussed in a review of the literature (1).

Physical Examination

Spider angiomata and palmar erythema are common during pregnancy and usually disappear after delivery. In late pregnancy, physical examination of the liver is difficult because of the expanding uterus.

Ultrasonographic Examination

Ultrasonographic examination reveals no dilatation of the biliary tract, but increases in fasting gallbladder volume and residual volume after contraction are noted.

Pathology

Standard and ultrastructural examination of the liver during normal pregnancy reveals no or minimal abnormalities.

Hemodynamics

The plasma volume increases steadily between weeks 6 and 36 of gestation (by approximately 50%). The red cell volume also increases, but the increase is moderate (approximately 20%) and delayed. Consequently, the total blood volume increases, with hemodilution reflected by a decrease in the hematocrit value. It is necessary to bear this phenomenon of hemodilution in mind during the interpretation of all the serum concentrations during pregnancy. The plasma volume and red cell volume decrease rapidly after the termination of pregnancy, aided by the loss of blood at delivery. Cardiac output increases until the second

trimester and then decreases and normalizes near term. Absolute hepatic blood flow remains unchanged, but the percentage of cardiac output to the liver decreases.

Serum Protein and Lipids

The serum albumin levels decrease during the first trimester, and this decrease becomes more accentuated as the pregnancy advances. However, the serum concentrations of some proteins increase, such as α_2-macroglobulin, ceruloplasmin, and fibrinogen. The serum cholesterol and triglyceride concentrations increase markedly during pregnancy, and except when a pregnant woman is suffering from acute pancreatitis, the measurement of these serum lipid concentrations is rarely useful during pregnancy. The prothrombin time is unchanged during pregnancy.

Liver Tests

Knowledge of the changes associated with normal pregnancy is necessary for the interpretation of liver test values and the management of liver diseases during pregnancy (1,2). The serum alkaline phosphatase levels increase late in pregnancy, mainly during the third trimester, as a result of the production of the placental isoenzyme and an increase in the bone isoenzyme level. In most published studies, the serum levels of alanine transaminase (ALT) and aspartate transaminase (AST) have been found to remain within normal limits during pregnancy. Serum γ-glutamyl transpeptidase (GGT) activity decreases slightly during late pregnancy. The total and free bilirubin concentrations are lower than those in nonpregnant controls

during all three trimesters, as are the concentrations of conjugated bilirubin during the second and third trimesters. The serum 5′-nucleotidase activity is normal or slightly higher during the second and third trimesters compared to that in nonpregnant women. Fasting serum total bile acid (TBA) concentrations usually remain within normal limits, and their routine measurement remains useful for the diagnosis of cholestasis during pregnancy, especially when routine liver function test results are still within normal limits. Therefore, increased values of serum ALT and AST activity, and serum bilirubin and fasting TBA concentrations should be considered pathologic, as they are in nonpregnant women, and prompt further evaluation. The main changes in liver function test results during normal pregnancy compared to nonpregnant women are summarized in Table 46.1.

Table 46.1. Liver Tests in Normal Pregnancy

TESTS NOT AFFECTED BY PREGNANCY
Serum transaminase levels (alanine transaminase, aspartate transaminase)
Prothrombin time
Serum concentration of total bile acids (fasting state)
TESTS AFFECTED BY PREGNANCY[a]
Albuminemia (decreased from the first trimester)
Alkaline phosphatase levels (increased in second and above all in third trimester)

Bilirubinemia (slightly decreased from the first trimester) 5′ nucleotidase (slightly increased) γ-Glutamyltransferase (slightly decreased in late pregnancy)

[a]Increased or decreased in relation to values in nonpregnant women.

Liver Diseases Unique to Pregnancy

Liver diseases unique to pregnancy include primary hepatic pregnancy, hyperemesis gravidarum, intrahepatic cholestasis of pregnancy (ICP), the liver disorders of preeclampsia with HELLP syndrome, and acute fatty liver of pregnancy (AFLP). The main factors in the diagnosis of liver disease in pregnancy are given in Table 46.2. Gestational age at the time of the onset of signs and symptoms can be helpful in the differential diagnosis. Hyperemesis gravidarum begins in the early part of the first trimester. ICP can begin at any time but usually does not present until the second or third trimester. Preeclampsia is a disorder of the second half of pregnancy, and patients with HELLP syndrome usually present in the third trimester. Similarly, AFLP, which can be associated with preeclampsia, is usually a disorder of the third trimester of pregnancy. The main diagnostic features of these liver diseases are given in Table 46.3.

Table 46.2. Main Factors in the Diagnosis of Liver Diseases in Pregnancy

Jaundice Generalized pruritus Nausea or vomiting Pain in epigastrium or right hypochondrium Arterial hypertension and proteinuria Polyuria and polydipsia without diabetes mellitus Thrombocytopenia

Primary Hepatic Pregnancy

On exceedingly rare occasions, the inferior surface of the right lobe of the liver is the site of ectopic implantation (Fig. 46.1). Such patients may present early in gestation with hemoperitoneum resulting from hepatic hemorrhage. If the

pregnancy progresses toward term, the patient presents with a mass in the liver. Primary hepatic pregnancy can be diagnosed by ultrasound examination or computed tomography scan (3), and termination of pregnancy by laparotomy is recommended in view of the risk of rupture.

Hyperemesis Gravidarum

Nausea and vomiting are common symptoms of early pregnancy, occurring in up to 50% of all pregnancies, corresponding to "morning sickness." By contrast hyperemesis gravidarum occurs much less frequently, complicating approximately 0.5% to 1.5% of pregnancies (4,5). There is no clear demarcation between common symptoms and severe forms, and therefore, there is no universally accepted definition. Hyperemesis gravidarum can be defined as persistent vomiting associated with weight loss greater than 5% of prepregnancy body weight and large ketonuria (5). Hyperemesis gravidarum leads to dehydration, and hospitalization is usually required. Liver involvement, as described in the subsequent text, is common in this condition (6).

Pathology

Liver biopsy is rarely needed to confirm this diagnosis, given its typical clinical presentation. When performed, it shows surprisingly little. There is no inflammation, but centrilobular vacuolization, necrosis with cell dropout, and rare bile plugs may be seen (7).

Clinical and biochemical findings

This disorder presents early in the first trimester of pregnancy, during weeks 4 to 10 of gestation, with intractable vomiting and associated ptyalism. As a rule, it resolves by the 20th week, regardless of therapy. It is more common during a first pregnancy than in multiparous women. In the modern era, liver disease

is inconspicuous and jaundice is rare. This was not the case before the widespread use of intravenous fluids; for example, Charlotte Brontë, the author of Jane Eyre, died in 1855 with nausea, vomiting, and jaundice during the fourth month of her first pregnancy.

Table 46.3. Main Diagnostic Features of Liver Diseases in Pregnancy

QUESTIONING
Term of pregnancy, past medical history with emphasis on the history of pruritus during previous pregnancy or oral contraception, pruritus during current pregnancy, abdominal pain, nausea or vomiting, polyuria and polydipsia, drug treatment

CLINICAL EXAMINATION
Temperature, blood pressure, liver examination (difficult during late pregnancy), herpetic vesicles on skin or mucosa

BLOOD TESTS
Routine liver function tests (Table 46.1; liver function tests in normal pregnancy)

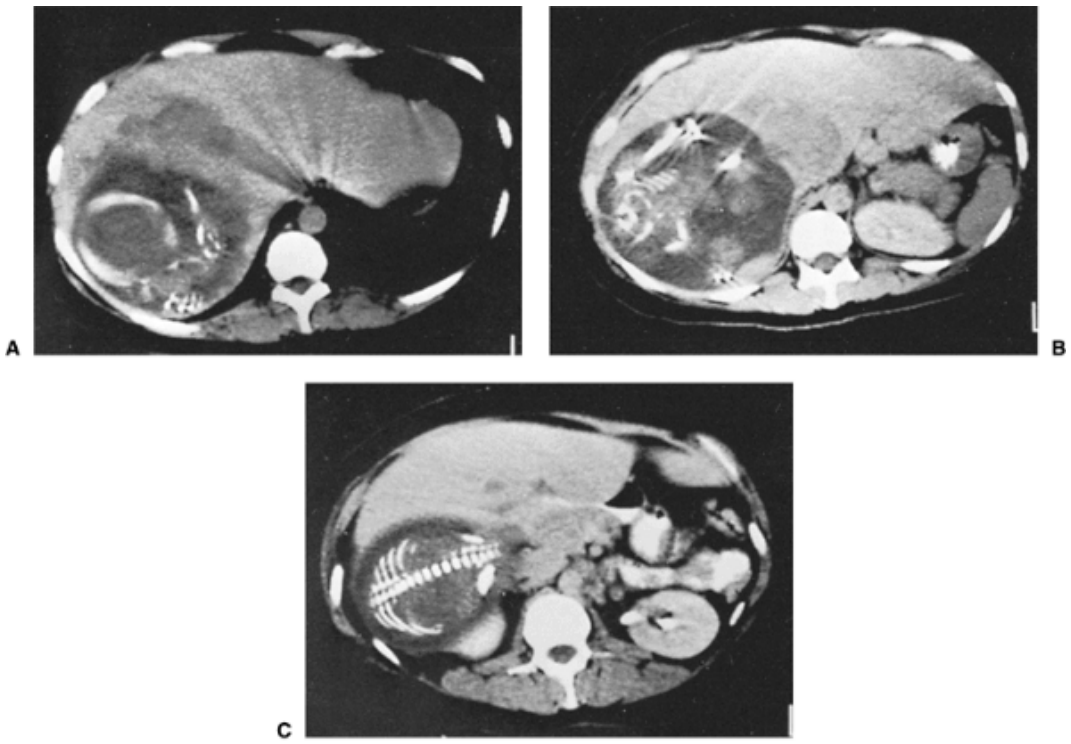
Glycemia, creatininemia, electrolytes and uricemia
Full blood count including platelets
Prothrombin time
Viral hepatitis and cytomegalovirus serology
Possible measurement of serum total bile acid levels when cholestasis is suspected (not a routine test)

URINE TESTS

Proteinuria and bacteriuria

ULTRASONOGRAPHY OF THE LIVER AND BILE DUCTS

EVOLUTION OF SYMPTOMS AND LIVER FUNCTION TESTS AFTER DELIVERY



▪ **Figure 46.1** Intrahepatic pregnancy demonstrated by computed tomography. **A:** A cut from below the dome of the liver showing skull bones of the fetus and the placenta invading the hepatic substance. **B, C:** Lower cuts demonstrating fetal position with the spine protruding from the inferior surface of the liver. (Permission of Professor Caroline A. Riely.)

When the liver is involved, the most striking abnormality is elevation of aminotransferase levels, with ALT levels exceeding AST levels and both readings usually in the low hundreds but rarely as high as 1,000 IU. Increases in bilirubin levels occur but are less striking. When the patient is treated with gut rest and intravenous fluids, the abnormalities resolve. Pregnancies complicated by hyperemesis gravidarum have been associated with transient hyperthyroidism, and an association between the liver involvement and the hyperthyroidism is

possible (8).

Affected patients may be thought to have hepatitis or gastric outlet obstruction resulting from peptic ulcer disease. Hepatitis serologies are useful in the differential diagnosis. Abdominal pain is not a typical complaint.

Maternal and fetal outcome

Many affected patients respond to rehydration and a short period of gut rest followed by reintroduction of a diet rich in carbohydrates and low in fat. Thiamine supplementation may be recommended for women who have vomited for several weeks to prevent Wernicke's encephalopathy. Antiemetics including promethazine, metoclopramide, ondansetron, and droperidol may be useful (9,10). Corticosteroids are reported to improve the appetite and have been proposed in this condition (11,12). Enteral nutrition through gastric or duodenal intubation is effective and preferable to the parenteral route (13). Despite the severity of the illness and attendant weight loss, infants born after affected pregnancies do not differ in regard to birth weight, gestational age, and birth defects from infants born after pregnancies unaffected by hyperemesis gravidarum (4,14).

Pathophysiology

The pathogenesis of this disorder remains unclear, but liver involvement, like thyroid involvement, appears to be secondary to the disorder itself, not a causative factor. Infection with *Helicobacter pylori* may play a role (15), and a predominance of female offspring in affected pregnancies has been noted (16). It is presumed that gestational hormones, many of which peak in the early part of pregnancy, affect both the liver and the thyroid.

Intrahepatic Cholestasis of Pregnancy

ICP occurs during the second or third trimester and disappears spontaneously after delivery. The prevalence of ICP varies widely by country. It is common in Scandinavia and even more common in Bolivia and Chile. In Chile, the prevalence in 1974 to 1975 was reported to be 15.6%, ranging from 11.8% to 27.7%, according to ethnic origin (17). For unknown reasons, the prevalence has more recently appeared to decrease (to between 4.0% and 6.5%) (18,19). Generally, ICP is more common in twin pregnancies (20).

Pathology

Liver biopsy is rarely necessary for the diagnosis. Histopathology is characterized by pure cholestasis, sometimes with bile plugs in the hepatocytes and canaliculi, predominantly in zone 3. Inflammation and necrosis are not usually observed, and the portal tracts are unaffected (21).

Clinical and biochemical findings

Pruritus, which is the main symptom, is very uncomfortable and difficult to tolerate. It is often generalized but predominates on the palms and soles. It is more severe at night and disturbs sleep. Pruritus usually disappears within the first few days of delivery. The clinical examination findings are normal except for evidence of scratching. Fever, if present, is usually caused by an associated urinary tract infection. Approximately 10% to 20% of patients have jaundice. The greater frequency of jaundice in some studies may be a consequence of

concomitant urinary tract infection (22). ICP with jaundice but without pruritus is rare. Patients do not experience abdominal pain or encephalopathy. Ultrasonographic examination reveals no dilatation of the biliary tract.

Measurement of serum ALT activity is a sensitive test for the diagnosis of ICP. Patients with ICP frequently exhibit significant increases in serum ALT activity that suggest acute viral hepatitis, which should be ruled out with suitable serologic tests (23). Liver histology usually does not reveal necrotic lesions, and the ALT level elevations may be secondary to an increase in membrane permeability. The serum GGT activity is normal or only slightly increased, the serum 5'-nucleotidase activity is often slightly increased, and the serum TBA concentrations are increased (24). A relationship between maternal serum bile acid levels and fetal distress has been found (25,26) and evaluation of the serum TBA concentration has been recently suggested as a means of fetal assessment in patients with ICP (27). At the present time, however, no consensus has been reached concerning the usefulness of evaluating the serum TBA concentrations in the obstetric management of patients with ICP (28). Little or no correlation has been found between the serum TBA concentrations and other liver test values (23). The serum bile acid concentration and serum ALT activity decrease rapidly after delivery and, as a rule, normalize in a few weeks.

Recently, the measurement of serum glutathione-*S*-transferase, a maker of hepatocellular integrity, has been proposed to distinguish ICP from "benign pruritus gravidarum" (29). The prothrombin time is usually normal. It may become abnormal in severe cholestasis with jaundice or in patients who have been treated with cholestyramine. The abnormality is caused by vitamin K deficiency, which should be anticipated and treated before delivery to prevent hemorrhage. Such therapy contributes to a good maternal prognosis.

Maternal and fetal outcome

The maternal prognosis is good, but cholestasis frequently recurs in subsequent pregnancies. The administration of oral contraceptives to women with a history of ICP may rarely result in cholestasis, but ICP is not a contraindication for oral contraceptives. Oral estroprogestogen contraception with a low dose of estrogen can be initiated after the liver test values have normalized. The patient should be informed of the possibility of pruritus during such contraception.

ICP does carry a risk for the fetus (30). The main complication of ICP is prematurity, which is more frequent in patients with ICP than in the general population (19). The rate of prematurity varies greatly according to the study and may be increased because of the high rate of multiple pregnancies in patients with ICP (23). The other complication of ICP is the risk of sudden fetal death. The prevalence is approximately 1% to 2% but varies according to studies. Sudden fetal death rarely occurs before the last month of pregnancy.

Pathophysiology

The cause of ICP is unknown. The results of previous epidemiologic and clinical studies suggest that genetic, hormonal, and exogenous factors play a role. Genetic factors may explain the familial cases and the higher incidence in some ethnic groups, such as the Araucanos Indians of Chile (17). A nonsense mutation of the *ABCB4* (*MDR3*) gene has been found in a child with progressive familial intrahepatic cholestasis type 3 (PFIC 3) and in three mothers suffering from cholestasis during pregnancy (31). In this familial study, the infant with PFIC 3

was homozygous for the *ABCB4* mutations, whereas the mothers with ICP were heterozygous. The *ABCB4* gene codes for the translator of phosphatidylcholine across the canalicular membrane into bile. In the absence of phospholipids in bile, bile acids can injure the canalicular membrane, leading to cholestasis. Several other mutations of the *ABCB4* gene were subsequently found in patients suffering from ICP (32,33,34). However, the role of ABCB4 in the pathogenesis of ICP has not been clearly established and the prevalence of such mutations in patients with a clearly defined phenotype of ICP is still under evaluation (35,36). Defects in the *ATP8B1* gene, which is associated with progressive familial intrahepatic cholestasis type 1 (PFIC1) and benign recurrent intrahepatic cholestasis (BRIC), have also been found in patients with ICP, but it seems that this gene is not a major contributor to ICP (37). A role of estrogens has been clearly established in ICP. Animal studies have shown that estrogens, in particular ethynyl estradiol, are cholestatic. Genetically determined abnormalities may lead to unique hepatic reactions to estrogens or to dysfunction of estrogen metabolism (30). Progesterone metabolism is also involved in the pathophysiology. Abnormalities of progesterone metabolism, especially elevated levels of serum sulfated metabolites, have been found in women with ICP (38). The formation of large amounts of sulfated progesterone metabolites, possibly related to greater 5-α and 3-α reduction, may in some genetically predisposed women result in a saturation of the hepatic transport system(s) involved in the biliary excretion of these compounds (39). One study has shown that oral natural progesterone prescribed for threatened premature delivery can trigger ICP in predisposed women (23). The intake of progesterone may place an additional load on the sulfated metabolite transport system. Progesterone treatment should therefore be avoided in pregnant women, especially late in pregnancy or when the patient has a history of ICP.

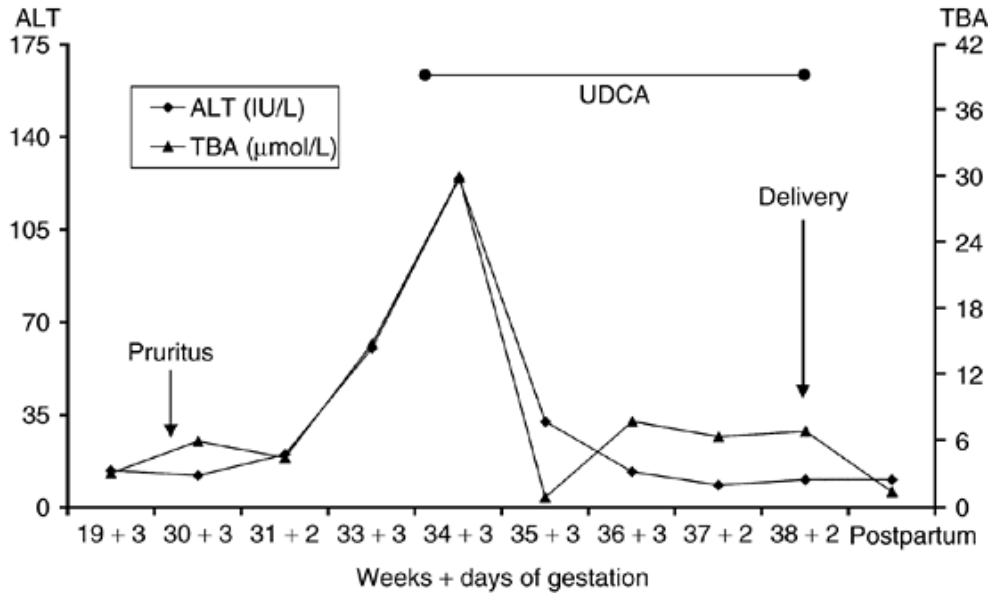
Some characteristics of ICP suggest that exogenous factors may be associated with an underlying genetic predisposition: (a) ICP recurs in only 60% to 70% of pregnancies in multiparous women, (b) seasonal variability has been observed in several countries, and (c) the prevalence of ICP has decreased in Sweden and Chile. For example, a deficiency in selenium may be a factor involved in the pathophysiology of ICP (40).

Medical and obstetric management

Hydroxyzine (25 to 50 mg/day) may alleviate the discomfort of pruritus. Cholestyramine (8 to 16 g/day) decreases the ileal absorption and increases the fecal excretion of bile salts. Its effect on pruritus is limited. The efficacy of *S*-adenosyl-L-methionine is a matter of debate (18,41,42). The most promising treatment is ursodeoxycholic acid. In some case reports and several open and controlled trials, ursodeoxycholic acid has been effective in ICP (38,42,43,44,45,46). The bile acid patterns in meconium are influenced by cholestasis of pregnancy and are not altered by treatment with ursodeoxycholic acid (45,46). Ursodeoxycholic acid relieves pruritus, improves liver function test values (Fig. 46.2), and prevents prematurity. No side effects have been reported for mothers or babies. Therefore, ursodeoxycholic acid (usually 500 mg twice a day or 15 mg/kg per day) appears to be safe during late pregnancy and may be useful in relieving cholestasis

and improving fetal prognosis in patients with ICP, especially those with severe disease. The mechanism of the beneficial effect of ursodeoxycholic acid for the mother and the baby in ICP remains speculative. Like in chronic liver diseases,

ursodeoxycholic acid, which is a hydrophilic bile acid, may decrease signs of cholestasis in the mother by affording cytoprotection against the hepatotoxic effects of the hydrophobic bile acids and by improving the hepatobiliary bile acid transport. In ICP, ursodeoxycholic acid may also have a specific effect by improving the transport of bile acid across the placenta.



▪ **Figure 46.2** Treatment of intrahepatic cholestasis of pregnancy (ICP) with ursodeoxycholic acid (UDCA). This patient had experienced ICP during a previous pregnancy. Regular surveillance during the current pregnancy was proposed and once the diagnosis of recurrent ICP had been confirmed treatment with UDCA was initiated. Serum total bile acid (TBA) concentration (TBA, upper normal limit: 6 µmol/L) and serum alanine aminotransferase (ALT) activity (ALT, upper normal limit: 35 IU/L) improved and the patient delivered at 38 weeks of gestation. (Yannick Bacq, personal data, 2005)

It is often difficult to decide the best time for delivery, and no consensus has been clearly established. When cholestasis is severe (e.g., if the patient has clinical jaundice), delivery should be considered at 36 weeks of gestation if the fetal lungs have matured, or as soon thereafter as possible (19).

Preeclampsia Liver Disorders

Livers disorders associated with preeclampsia are certainly the most frequent causes among the liver diseases unique to pregnancy (47). Preeclampsia is a multisystem disorder of enigmatic etiology and pathogenesis presenting in the latter half of pregnancy (48,49). It complicates 3% to 5% of all pregnancies and is a major cause of maternal and fetal mortality. Despite the importance of this disorder, even its definition is still debated (50). The disease is thought to start early in pregnancy, with abnormal implantation of the trophoblast, which leads to restricted perfusion of the placenta. The fall in systemic vascular resistance typical of normal pregnancy does not occur in patients with preeclampsia; their sensitivity to vasospasm is enhanced, with resultant poor perfusion of and injury to a variety of organs, including the liver. A variety of factors is suspected to

play an important role in the mechanism of the disease, some inherited, some immunologic. The disorder is more common in primiparas and in multiple gestations with twins or triplets (51,52). It is also more common in multiparous women whose first pregnancy was complicated by preeclampsia (53). Being the offspring of either a father or a mother who was born of a preeclamptic pregnancy increases the risk of preeclampsia in the next generation (54). A possible role for an inherited procoagulant state was suggested (55,56,57), but then refuted (58). A link to insulin resistance syndrome is possible (59,60). Abnormalities in endothelial function, probably mediated by nitric oxide, can be demonstrated in women with a history of preeclampsia (61). Antioxidant treatment was shown to decrease the incidence of preeclampsia in susceptible women (62), which suggests a role for oxidative stress in this condition (63). Preeclampsia may result from paternal or fetal effects, and immune factors may play a role (48). An excess of male offspring from affected pregnancies has been noted (64). Preeclampsia is more common in couples who have cohabited for only a short period (65) and in multiparous women who then become pregnant by a different partner (66).

The typical presentation is hypertension with proteinuria, although both conditions are not found in all patients with this multisystem disease. Patients may also have renal failure, seizures (eclampsia), pancreatitis, or pulmonary edema. Preeclampsia has long been

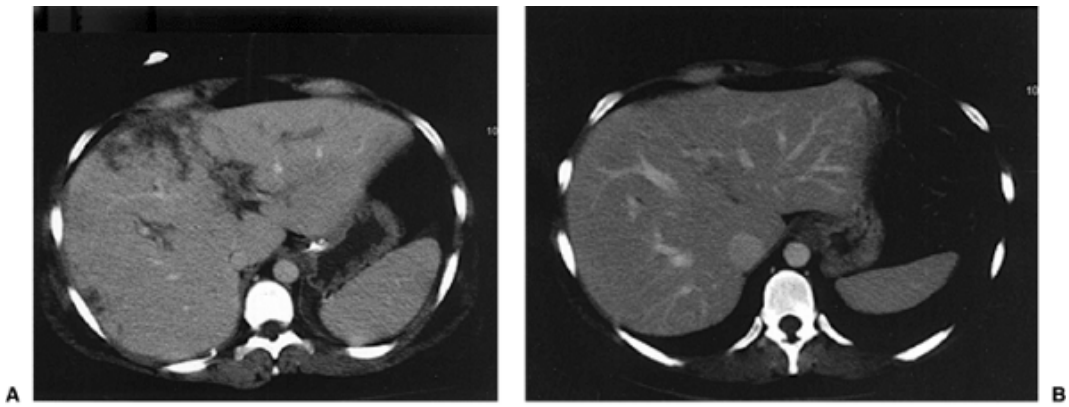
known to affect the liver in a variety of ways including HELLP syndrome.

Hemolysis, elevated liver enzymes, low platelets syndrome

The HELLP syndrome is defined as hemolysis (usually subclinical, with characteristic schistocytes and burr cells on smear), elevated liver enzyme levels (usually elevated aminotransferase levels, with AST exceeding ALT), and low platelet numbers in a patient with preeclampsia. Affected women are less likely to be primiparous and tend to be older than the average woman with preeclampsia (67). The hepatic histology is that of preeclamptic liver disease, with periportal hemorrhage and fibrin deposition. Little correlation is found between the degree of histologic aberration and the severity of the clinical findings. Fat may be seen, but as macrovesicular fat distributed in modest quantities throughout the liver lobule and not as the microvesicular centrizonal fat typical of AFLP (68). Despite similar settings and occasional clinical overlap, these two conditions are histologically distinct (69).

The clinical presentation of HELLP varies markedly, with no symptom other than abdominal pain recorded in more than 50% of patients (53). The pain is usually located in the midepigastric region, right upper quadrant, or substernal region. Many patients have nausea, vomiting, and malaise, which suggest a diagnosis of viral hepatitis. Jaundice is present in approximately 5% of patients (53). Most cases are diagnosed during the third trimester, although the condition may present postpartum. In a large series of 437 patients who had 442 pregnancies with HELLP syndrome, 70% of cases occurred before delivery and 30% after delivery, 11% developed before 27 weeks of gestation and 18% after 37 weeks (53). Most, but not all, patients have the hypertension and proteinuria that are typical of preeclampsia. The diagnosis usually rests on clinical grounds, although imaging studies, particularly computed tomography (Fig. 46.3) and magnetic resonance imaging, are useful in detecting the complications of hepatic infarct,

hematoma, and rupture (70). Hepatic hematoma may be missed by these techniques but detected laparoscopically (71). Liver biopsy should be approached with caution, given the association of hematoma and rupture with HELLP.



▪ **Figure 46.3** Hepatic involvement in a 24-year-old primipara with severe preeclampsia. **A:** Abdominal computed tomography performed after delivery by cesarean section showing subcapsular ischemic lesions in the right lobe. **B:** Follow-up image 7 months later showing complete recovery. (Courtesy of Béatrice Scotto, MD.)

The outcome for the mother is usually good, with a maternal mortality rate of 1.1% in a large series (53), and the condition starts to reverse with delivery. A review of deaths collected from many centers demonstrated that stroke is the most common cause of death, followed by cardiac arrest and disseminated intravascular coagulation (72). No correlation between any laboratory test value (e.g., platelet count, or aminotransferase or lactate dehydrogenase level) and adverse maternal outcome was reported in another study (73). Rare patients experience severe liver disease with fulminant hepatic failure leading to death (74). Diabetes insipidus has been reported in HELLP syndrome and also in AFLP (75). The main risk for the fetus is prematurity. These babies do not have any increased risk of liver disease or thrombocytopenia, and their outcome is similar to that of babies of a similar gestational age (76,77).

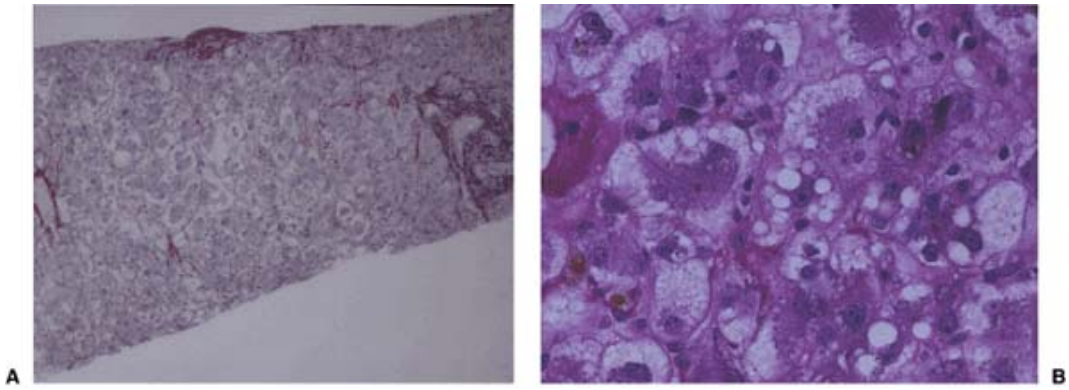
Management is supportive and may include transfer to a medical intensive care unit, with ventilatory support and dialysis provided in severe cases. The cornerstone of therapy is delivery, although temporizing management with intensive monitoring may be useful for some patients with mild disease (78). Corticosteroids have been shown to improve laboratory test

values and allow a delay in delivery (79,80,81). Long-term follow-up has shown an increased risk of obstetric complications in subsequent pregnancies but no tendency for a repetition of the HELLP syndrome (67).

Hepatic hematoma and rupture

In rare pregnant patients, a hematoma develops beneath the Glisson capsule. This may remain contained, or rupture of the liver capsule may result in hemorrhage into the peritoneal cavity from multiple lacerations in which the

capsule has been lifted from the surface. Rupture usually occurs in the setting of HELLP syndrome. Histology of the portion of the liver adjacent to the rupture shows periportal hemorrhage and fibrin deposition, along with neutrophil infiltrate, suggestive of hepatic preeclampsia (53,82). Some patients do not have thrombocytopenia or typical preeclampsia (83). Clinically, affected patients have abdominal pain and, when the liver has ruptured, swelling of the belly from hemoperitoneum, along with shock. The aminotransferase levels are usually slightly raised, but values in the range of 4,000 to 5,000 IU are occasionally seen. Computed tomography or magnetic resonance imaging of the body is more dependable than ultrasonography for detecting these lesions (70). The management of a contained hematoma is supportive. Patients with rupture are best managed by a team experienced in liver trauma surgery (84). Liver transplantation is used when the hemorrhage cannot be contained (85,86,87). Patients who survive have no hepatic sequelae and have been documented to have normal subsequent pregnancies (67). A report of recurrent episodes in subsequent pregnancies suggested a predisposition of affected women, resulting from some underlying condition, perhaps an inherited procoagulant state (88).



▪ **Figure 46.4** Acute fatty liver of pregnancy in a 24-year-old primipara. This woman had experienced nausea and vomiting, abdominal pains, and jaundice in late pregnancy. A transvenous liver biopsy was performed postpartum—**(A)** intact lobular architecture (hematoxylin and eosin, 200×) and **(B)** typical microvesicular fatty infiltration in hepatocytes and features of cholestasis (400×). (Courtesy of Anne de Muret, MD.)

Acute Fatty Liver of Pregnancy

AFLP was distinguished as a specific clinical entity unique to pregnancy in 1940 (89). It is a rare disease and its incidence was estimated to be 1 per 13,328 deliveries at the Los Angeles County University of the Southern California Medical Center (90) and 1 per 15,900 in Santiago, Chile (91). Early diagnosis and prompt delivery have dramatically improved both maternal and fetal prognosis, as perhaps has the recognition of the disease, and an article from Los Angeles County states the incidence to be 1 in 6,659 births (92). In addition, in a recent prospective study including 4,377 deliveries in Southwest Wales, AFLP was found in five patients (i.e., 1 per 875 deliveries) (47).

Pathology

Liver biopsy is the best way to confirm the diagnosis of AFLP, but because it is invasive, it is not always performed. Also, we can now take advantage of noninvasive procedures to demonstrate fat in the liver and exclude other liver diseases, such as viral hepatitis. Nevertheless, liver biopsy may be useful in atypical cases.

The overall architecture of the liver is not altered. The characteristic picture is a microvesicular fatty infiltration of the hepatocytes, which are swollen. The droplets are minute and surround centrally located nuclei, so that the cytoplasm has a foamy appearance (Fig. 46.4). In a few cases, rare, large fat vacuoles are associated with the microvesicular steatosis. The microvesicular fatty infiltration is most prominent in the pericentral zones and midzones (zones 2 and 3) and usually spares a rim of periportal cells. The droplets stain with oil red O, which is specific for fat. The histologic features of cholestasis (i.e., bile thrombi or bile

deposits within hepatocytes) are common. Inflammation is not prominent but is also common (93). The histologic features are not always evident, and cases of AFLP have been misdiagnosed as hepatitis. Necrosis with acidophilic bodies is inconspicuous, and massive panlobular hepatocellular necrosis, as in fulminant viral hepatitis, is not seen. On the other hand, the liver may appear disorganized, with impressive lobular disarray and pleomorphic hepatocytes (94). Electron microscopy confirms the presence of fat droplets and has shown nonspecific changes in mitochondrial size and shape (95). A stain specific for fat or electron microscopy is useful for pathologic confirmation of the diagnosis in patients with ballooning of the cytoplasm but no evident vacuolization. Therefore, whenever AFLP is suspected, a piece of the liver biopsy specimen should be reserved before paraffin embedding and processed appropriately with special stains to confirm the presence of fat in the hepatocytes. The pathologic changes normally reverse rapidly after delivery, and AFLP is not associated with progression to cirrhosis (94).

Clinical and biochemical findings

As a rule, AFLP is a disease of the third trimester that may occur during any period of gestation. However, some reports have detailed cases presenting at 22 (96) or 26 weeks of gestation (97). The onset of disease is never after delivery, but the diagnosis may be made after delivery. The frequency of twin gestations is increased among patients with AFLP (14% to 19% vs. approximately 1% in the general population), and 7% of triplet pregnancies have been reported to be complicated by AFLP (52). The most frequent initial symptoms are nausea or vomiting, abdominal pain (especially epigastric), anorexia, and jaundice. In the past, jaundice was almost always seen during the course of the disease, but because of earlier diagnosis, prompt delivery, and the diagnosis of milder cases, we now see affected patients without jaundice. The size of the liver is usually normal or small. Patients with AFLP rarely have pruritus but may have concurrent ICP (98). Approximately half of affected patients have high blood pressure or proteinuria, which are the main symptoms of preeclampsia (93). On the other hand, some affected patients do not have any of these signs. Patients may demonstrate asterixis and encephalopathy, with or without coma, and some have pancreatitis. Esophagitis and Mallory-Weiss syndrome related to severe vomiting have been reported. Gastrointestinal bleeding secondary to the esophageal lesions, in addition to gastric ulceration related to shock, has been reported.

Genital bleeding is frequent. These hemorrhages are exacerbated by associated coagulation disorders. Ascites may be present and is partially related to portal hypertension (91). Polyuria and polydipsia have been noted in about 5% of patients with AFLP (93), and an association between transient diabetes insipidus and AFLP has been reported (99).

The serum aminotransferase levels are raised, but usually the level is not as high as that in acute viral hepatitis. The bilirubin level is almost always increased. Patients may demonstrate hypoglycemia. In severe cases, the prothrombin time is increased and the fibrinogen level decreased. These coagulation disorders are caused by hepatic insufficiency, disseminated intravascular coagulation, or both. A low platelet count is usual in AFLP and is not always associated with other signs of disseminated intravascular coagulation. Thrombocytopenia may be the most striking laboratory feature and normalizes spontaneously after delivery. The diagnosis of AFLP should always be considered when thrombocytopenia occurs during late pregnancy and should always prompt the performance of liver function tests, particularly determination of the aminotransferase levels. Renal failure (mainly functional) and hyperuricemia are usual. Ultrasonography of the liver may show increased echogenicity (100). Computed tomography may be useful for the diagnosis, and a liver density lower than usual may be demonstrated by Hounsfield unit values in the liver that are equal to or lower than those in the spleen (101). The findings on imaging studies may be normal; a recent study showed that the findings on computed tomography, which is more sensitive than ultrasonography, were normal in half of the patients with AFLP (102). In clinical practice, these complementary examinations should not delay delivery, particularly in severe cases, which can usually be diagnosed on clinical grounds with routine biologic data.

Maternal and fetal outcome

The maternal mortality rate of AFLP was very high before 1970 (approximately 90%) (93). The maternal prognosis has currently improved greatly, and maternal mortality is less than 10%. This improvement is principally related to early delivery, advances in intensive care support for patients with severe forms, and also the detection of patients with less severe forms. Most recover completely after delivery, without sequelae. However, one patient remained in prolonged coma after hemorrhagic shock (103), and cases of neurohypophyseal insufficiency have been reported, which, in one case has been associated with definitive diabetes insipidus (104).

AFLP may recur during subsequent pregnancies, although recurrence is not the rule. At least 25 cases without recurrence have been reported; 17 patients had a normal pregnancy and 4 had two normal pregnancies after AFLP. However, at least six cases of recurrence have been reported since 1990. In the first case, both

episodes of AFLP were confirmed by histologic examination of the liver biopsy specimen, and the mother and babies recovered (105). In the second case, although healthy children were delivered by cesarean section, both babies died at 6 months (106). Steatosis of the liver was present in these neonates and associated with a deficiency of β-oxidation of fatty acids. The mother had a third pregnancy, which was uneventful, and the infant did not have such a deficiency. In the third case, the baby died in utero during the first episode of AFLP (91). During the recurrence 5 years later, emergency cesarean section performed at 36

weeks resulted in a live birth, and the outcome was good for both mother and baby. The outcome was favorable for mothers and babies in three other cases of recurrence reported in the literature. Mothers who have experienced AFLP should be informed of the risk for recurrence and closely followed up during subsequent pregnancies. Follow-up should be both clinical and biologic (e.g., liver function tests, tests for uricemia, and platelet counts twice monthly during the third trimester).

Until 1985, fetal mortality was reported to be as high as 50% (94). Early delivery has resulted in an improved fetal prognosis (107), and the final outcome for infants delivered alive is usually considered to be good. However, in view of the possibility of congenital enzyme deficiency involving intramitochondrial β-oxidation of fatty acids, these infants should be closely followed up from birth.

Pathophysiology

AFLP belongs to a group of liver diseases characterized by microvesicular steatosis, including Reye's syndrome, sodium valproate and tetracycline toxicity, and Jamaican vomiting sickness. All these conditions are considered to be caused by abnormalities of mitochondrial function. Microvesicular steatosis may occur in several other drug-induced, toxic, and viral liver diseases.

The cause of AFLP remains unknown, although good progress toward a better understanding of this disorder has recently been made. An association of inherited defects in the β-oxidation of fatty acids with AFLP is now well established. A case of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency in a 4-month-old baby born to a mother who had had AFLP and HELLP syndrome at 36 weeks of pregnancy was reported in 1994 (108). Both parents were heterozygous for LCHAD deficiency. Hepatic steatosis or HELLP syndrome has also been reported in several mothers of babies with LCHAD deficiency (109,110). β-Oxidation of fatty acids, measured by the activity of LCHAD in skin fibroblast culture, was studied in 12 women who had had AFLP (110). LCHAD activity was reduced in eight of them, consistent with heterozygosity for LCHAD deficiency. Four women had no deficiency. The eight heterozygous women had a total of nine pregnancies complicated by AFLP. Of the nine offspring delivered from these pregnancies, four were confirmed to be homozygous for LCHAD deficiency. Three other infants died with a clinical picture compatible with this diagnosis. The two other infants were healthy at 18 and 24 months of age and had LCHAD activity in the heterozygous range. The five husbands tested were heterozygous (110). These findings show that a deficiency in the β-oxidation enzyme in the fetus may lead to maternal hepatic steatosis in late pregnancy, especially if the mother is heterozygous for the deficiency. Two mutations (G1528C and C1132T) have been observed in the gene coding for LCHAD in three families with children having LCHAD deficiency whose mothers had had AFLP or HELLP syndrome (111). More recent work shows that acute fatty liver may occur regardless of the mother's genotype if her fetus is deficient in LCHAD and carries at least one allele with the G1528C mutation (112). In affected families, a prenatal diagnosis based on sampling of chorionic villi has proved both feasible and accurate (113). These forms of AFLP associated with a genetic deficiency of β-oxidation have not been observed in all countries; the common mutation (G1528C) was not found in 14 women with AFLP observed consecutively in a French hospital (114). Another defect in β-oxidation, a deficiency of carnitine palmitoyltransferase I, has also been associated with AFLP (115), and DNA analysis for this deficiency is available (116). No familial cases (i.e., AFLP in

mother and daughter) have been reported.

Medical and obstetric management

AFLP must be considered an obstetric emergency. AFLP usually does not resolve before delivery, and if delivery is delayed, complications such as hemorrhage and intrauterine death may develop. Consequently, the primary therapy for AFLP is early delivery. The choice of the route of delivery remains the decision of the obstetrician and must be appropriate for the individual clinical situation. Generally speaking, if the patient is in labor and in good general condition and no sign of fetal distress is detected, then vaginal delivery may be attempted with careful monitoring of the mother and baby (117). For patients with severe disease, urgent delivery must be considered, usually by cesarean section, after correction of the coagulation disorders, especially those related to thrombocytopenia.

The etiology of AFLP is not known, and no specific medical treatment is available. Esophagitis should be treated with appropriate drugs to prevent bleeding. The blood sugar levels should be monitored and

hypoglycemia treated by a continuous intravenous infusion of glucose. Patients with fulminant hepatic failure are best managed in an intensive care unit before and after delivery; intensive care remains the cornerstone of management. Two patients with serious disease that continued after delivery (which is unusual) were treated successfully by liver transplantation (118,119), and auxiliary transplantation has been used successfully in this setting (120). Nevertheless, the role of liver transplantation in AFLP is probably limited. Early diagnosis and prompt delivery of the infant of a patient with AFLP should avoid the difficult decision about later liver transplantation.

Intercurrent Liver Disease in Pregnancy

In addition to the disorders unique to pregnancy, pregnant women are susceptible to diseases that can affect anyone. Some common disorders can take a fulminant course in pregnant women, hepatitis E being the most frequent example. Furthermore, pregnancy predisposes a woman to the development of the usual liver diseases, such as cholelithiasis.

Acute Viral Hepatitis

The response of a pregnant woman to acute infection with the viruses that cause hepatitis varies, depending on the virus.

Hepatitis A

Pregnant women who contract hepatitis A are not at increased risk of severe disease from this infection (121), although the risk for premature labor may be increased in women who are seriously ill during the third trimester (122).

Hepatitis B

In patients with documented acute hepatitis B, pregnancy is not associated with increased mortality (123) or teratogenicity (124). Infection during gestation should not prompt termination of the pregnancy. Women exposed to hepatitis B during gestation may be vaccinated without any reported increase in congenital

anomalies (125); the vaccine is immunogenic in this setting (126).

Hepatitis E

This infection occurs both in epidemics and sporadically in many parts of the world (e.g., India, Pakistan, northern Africa, and Mexico). Women in the third trimester are more likely to have clinical disease than are other persons. The fatality rate in this group is as high as 25% (127,128,129). It can be difficult to distinguish fulminant hepatitis E from AFLP. Women in the third trimester of pregnancy should carefully consider the risks associated with travel to areas where this disease is endemic. Hepatitis E can cause acute hepatitis in the newborn and can be transmitted in utero to the fetus (127,129,130).

Hepatitis caused by herpes simplex virus and other viruses

When it occurs in the third trimester of pregnancy, hepatitis resulting from a primary systemic infection with herpes simplex virus is likely to be severe. Half of the reported cases of fulminant herpetic hepatitis have occurred in pregnant women (131). Affected patients may have a "viral" syndrome that includes fever and upper respiratory tract symptoms. Despite marked abnormalities in their aminotransferase levels and prothrombin time, these patients are usually anicteric at presentation. A vesicular eruption is diagnostically useful but may not yet be visible at presentation. Cultures and histology of liver biopsy specimens are helpful in the differential diagnosis, which should include severe liver disorders associated with pregnancy, such as AFLP and HELLP syndrome. Therapy with acyclovir is successful, and affected women need not be delivered of infants early. Acute infection with coxsackievirus B can cause a similar picture of acute hepatic failure (132).

Why hepatitis E and herpes simplex hepatitis are so much more severe and associated with such increased hepatic injury in the third trimester of pregnancy is unclear. Alterations in T-cell function have been reported in pregnancy and may be related to this enhanced susceptibility (133). Herpes simplex hepatitis is known to be more severe in certain immunocompromised states, such as chronic immunosuppression after transplantation.

Biliary Tract Disease and Pancreatitis

Pregnancy decreases gallbladder motility and increases the lithogenicity of bile (134). Pregnancy has long been considered a risk factor for the development of gallstones; epidemiologic studies confirm an association with an increased risk for gallstones, but only for a 5-year period after pregnancy. Thereafter, the risk drops back to that of the never-pregnant population (135). In adolescents with gallstones, a history of pregnancy is common (136). Ultrasonographic studies show that gallstones and biliary sludge may accumulate throughout gestation and resolve with a return to nonpregnant physiology (137,138). In a recent prospective study of 3,254 women, the cumulative incidence of new sludge, new stones, or progression of baseline sludge to stones

was 10.2%, by 4 to 6 weeks postpartum (vs. 5.1% by the first trimester). In the same study, 28 women (0.8%) underwent cholecystectomy within the first year postpartum (139).

Acute cholecystitis may occur in pregnancy. Operative cholecystectomy can be

performed during gestation, although it may increase maternal and fetal morbidity, particularly in the first trimester (140,141,142). In a large series of cholecystectomy in women, the procedure was only rarely performed during pregnancy (143), suggesting that acute cholecystitis, which is unresponsive to conservative medical management, is not a common occurrence during pregnancy. Recently, less invasive approaches to the problem of biliary lithiasis in pregnancy have been successful. Choledocholithiasis can be managed by endoscopic retrograde cholangiopancreatography with sphincterotomy (144,145). Laparoscopic cholecystectomy has also been reported to be successful in pregnancy (142,146), although the risk of morbidity may be increased (147).

Acute pancreatitis may complicate pregnancy. The serum amylase and lipase levels are normal during pregnancy, so abnormal values warrant attention (148). Pancreatitis usually occurs in the setting of cholelithiasis (149,150), and gallstones should be sought in any affected patient. Gallstone pancreatitis should be treated aggressively, either operatively (151) or endoscopically (144,152). Pancreatitis complicating pregnancy may be associated etiologically with AFLP or preeclampsia (153). Mild pancreatitis may occur in association with severe hyperemesis gravidarum, presumably as "refeeding pancreatitis." Familial hypertriglyceridemia, exacerbated by the physiologic hypertriglyceridemia of pregnancy, may present during pregnancy with pancreatitis (154). Hyperparathyroidism may be associated with pancreatitis in pregnancy (155,156).

A choledochal cyst may present during pregnancy, with abdominal pain, a mass, and jaundice (157). Such presentations may represent cases of congenital choledochal cyst exacerbated by the effects of pregnancy on biliary motility. Spontaneous rupture of a choledochal cyst (158) and of an apparently normal common hepatic duct (159) have been reported in pregnancy.

Hepatic Vein Thrombosis (Budd-Chiari Syndrome)

Both pregnancy and oral contraceptive therapy are associated with a hypercoagulable state (160). The frequency of hepatic vein thrombosis (Budd-Chiari syndrome) is increased in women using oral contraceptives (161). Reports from India suggest that it is also more common in pregnant women, usually manifesting immediately after delivery (162,163). Several reports have linked acute Budd-Chiari syndrome during pregnancy in western women with an underlying procoagulant state, such as primary antiphospholipid syndrome (164), anticardiolipin antibody (165), factor V Leiden mutation (166,167), or thrombotic thrombocytopenic purpura (168). The prognosis for pregnant women with this syndrome is ominous, as it is for pregnant women with idiopathic veno-occlusive disease. Liver transplantation has been used as a lifesaving measure (166,169), but such patients may survive with conservative measures, including delivery and anticoagulation. Recurrence has been reported in a patient whose anticoagulants were stopped when she became pregnant again (165). Nevertheless, subsequent successful, uncomplicated pregnancy has been reported for patients with a history of Budd-Chiari syndrome associated with oral contraceptives or an underlying myeloproliferative syndrome (170).

Drug-Induced Hepatic Injury

Because of concern about fetal teratogenicity, pregnant women in general take fewer drugs than those who are not pregnant. When they do take drugs, however, they run the same risk for adverse drug reactions as others. Potentially fatal hepatotoxicity has been reported in pregnant women undergoing

antiretroviral therapy for human immunodeficiency virus (HIV) infection (171). Acetaminophen overdose leading to death has been reported (172). On the other hand, no increased incidence of such adverse reactions during pregnancy has been documented. For example, 1,300 pregnant women took isoniazid for tuberculosis without ill effect (173).

Metastasis to the Liver

The liver is not palpable in normal pregnant women, and therefore, hepatomegaly detected on physical examination requires immediate evaluation. Patients with extensive tumor invasion of the liver may present with abdominal or back pain, rupture of the liver, or hepatic failure. The usual source is a common tumor, such as carcinoma of the colon (174,175) or pancreas (176). Gestational trophoblastic neoplasm (hydatidiform mole) can be a source of tumor spread to the liver. Breast cancer may also present with hepatomegaly during pregnancy. A patient presenting at 26 weeks of gestation with hypertension and thrombocytopenia, imitating HELLP syndrome, was found to have cholangiocarcinoma (177). It is possible that the modest immunosuppressive state associated with pregnancy promotes extensive tumor spread and growth.

Other Complicating Illnesses

Sepsis, particularly urinary tract infections, may be associated with jaundice in pregnant women (178),

as they are in the nonpregnant state. Echinococcal cysts of the liver have been reported during pregnancy (179,180).

Pregnancy in Women with Chronic Liver Disease

Monitoring of liver disease is necessary when its presence is known before pregnancy, and this requires cooperation between the obstetric team and hepatologists. Most patients with severe liver disease are not women of childbearing age or they are infertile because of the associated anovulatory state. Nevertheless, some of these women can become pregnant, and when they do, some special problems arise. On the other hand, most young women with chronic but nonsevere liver disease can have full-term pregnancies without any particular risk. However, there is the question of the effect of liver disease or its treatment on the fetus. Certain drugs should not be stopped during pregnancy because of the risk of relapse of the liver disease due to withdrawal of treatment. This is the case, for example, with immunosuppressive treatment of chronic autoimmune hepatitis and with penicillamine, which is used as a copper chelator in Wilson disease (see subsequent text). Other drugs, such as ribavirin used in the treatment of hepatitis C, are strictly contraindicated in pregnancy. In the case of ribavirin, the patient should be clearly informed of the need for effective contraception throughout treatment and during the 6 months thereafter.

Cirrhosis and Portal Hypertension

Worsening jaundice with progressive liver failure, ascites, and hepatic coma have been reported during the course of pregnancy in women with cirrhosis (181,182). Whether the exacerbation of hepatic dysfunction is caused by gestation or is merely coincident with it is unclear. What is clear, however, is that women with cirrhosis can often sustain pregnancy without any worsening of hepatic function (182). Published reports document an increased incidence of stillbirths and

premature delivery in such women (182,183,184).

The fertility of women with noncirrhotic portal hypertension, as seen in congenital hepatic fibrosis or portal vein thrombosis, is not diminished; therefore, pregnancy is encountered in this setting with some frequency (185,186). A worsening of preexisting portal hypertension may be anticipated because of the marked increase in blood volume and azygos flow that occurs during normal pregnancy. Variceal hemorrhage during pregnancy or labor has been reported (183,184,186,187). It is not clear whether the incidence of variceal hemorrhage in pregnant patients is higher than that in nonpregnant patients with known varices. Furthermore, a history of hemorrhage in one gestation does not predict the outcome of subsequent pregnancies (188). Sclerotherapy or endoscopic band ligation has been reported to be successful in pregnant women with variceal hemorrhage (185,189,190). Prevention of hemorrhage due to esophageal varices in women with known cirrhosis who desire pregnancy is based on classical treatment with β blockers and/or endoscopic ligature. An upper endoscopy is therefore usually performed before pregnancy. Prophylaxis with β blockers may be continued during pregnancy, but newborns should be monitored during the first days of life because of risks of hypoglycemia and bradycardia.

Specific Liver Diseases

Alcoholic liver disease

Alcoholic liver disease is often associated with infertility. However, most alcoholics do not have liver disease. Pregnancy in a fertile alcoholic woman can result in fetal alcohol syndrome in the infant, which includes typical facies, malformations, and developmental delay. Several such infants have been reported to have liver disease, with fatty liver and portal and perisinusoidal fibrosis suggestive of alcoholic liver disease (191). For the most part, however, liver disease or its treatment is not teratogenic. If they can succeed in getting pregnant, women with liver disease are not at increased risk of having children with congenital anomalies. On the other hand, they do have more maternal problems during pregnancy, and the risk of prematurity or stillbirth is greater.

Chronic hepatitis B

In general, pregnancy is well tolerated by women who are chronic carriers of the hepatitis B virus (HBV) (192); reactivation of the virus with exacerbation of disease during or after gestation is the exception rather than the rule (182,193). The placenta forms an excellent barrier against transmission of this large virus, and intrauterine infection with HBV is rare. It does occur, however, probably as a result of transplacental leakage, as in threatened abortion (194,195). The major problem for women who are chronic carriers of HBV is the risk of maternal-to-infant (vertical) transmission of infection at delivery. Transmission at birth is more likely if the mother is positive for

hepatitis B early antigen (HBeAg) (196) or has high circulating levels of HBV DNA (197). The rate of transmission may be lower if the delivery is by cesarean section (198). However, this is usually not indicated because appropriate immunoprophylaxis for the newborn with both HBV hyperimmune globulin and vaccine is efficient to interrupt transmission. Routine prenatal screening of all pregnant women for hepatitis B surface antigen (HBsAg) is now the standard of care (199). Infants born to infected mothers should be protected by the

recommended combination of vaccine and hepatitis B immunoglobulins at birth (199), although the emergence of mutant strains of the virus in such infants has been reported (200). Infants born to carriers of the HBV precore mutant who are positive for antibody anti-HBe and with high levels of HBV DNA in their serum are at risk for fulminant hepatitis B during the first 2 to 4 months after birth (201). Immunoprophylaxis should therefore be given to the infants of all mothers who are HBsAg positive, regardless of their HBe status. In women with very high HBV-DNA levels, vertical transmission of HBV can occur despite vaccination of the child. In eight such highly viremic HBsAg-positive women (HBV-DNA levels above 150pg/mL approximately equivalent to 1.2×10^9 geq/mL), a treatment with lamivudine (150 mg/day) during the last month of pregnancy decreased the HBV-DNA level and was associated with a reduction of the risk of child vaccination breakthrough (202). Hepatitis D (δ) virus can also be transmitted from mother to infant at birth (203).

Chronic hepatitis C

Uneventful pregnancy without worsening disease or fetal complications has been reported in women with hepatitis C (204). Several studies have demonstrated that aminotransferase levels decrease as the pregnancy progresses, whereas the HCV load, measured by polymerase chain reaction, increases during the course of gestation (205,206,207). Early evidence suggests that the hepatic histopathology may worsen during gestation (208). Whether pregnancy has any effect on the progression of this disease remains to be proved (209).

Transmission from mothers who are chronic carriers to their offspring may occur but appears to be much less efficient than the vertical transmission of hepatitis B (210). Rates of transmission were initially said to be proportional to the mother's viral burden, as indicated by the measurement of HCV RNA by polymerase chain reaction, and greatly enhanced transmission was supposed to occur in women coinfected with HIV, who have very high levels of viremia and rates of transmission ranging from 6% to 30% (211,212,213). More recent studies have not shown a correlation between a large viral load and increased maternal–infant transmission (205,207). Testing the newborn can be misleading. Early after birth, antibodies acquired from the mother result in positivity for HCV, and transient viremia can be seen in infants who later test negative. Cord blood can be negative for HCV RNA, although the infant is subsequently shown to be HCV RNA positive (205). An overall transmission rate of 5% according to testing performed 1 year after birth, regardless of the mother's HIV status, has been reported (205). No association with breast-feeding has been shown, and breast-feeding is not contraindicated (214,215). It may be prudent for mothers who are HCV infected and choose to breast-feed to consider abstaining from breast-feeding if their nipples are cracked and bleeding (215). The rate of transmission is not lower in infants delivered by cesarean section, and cesarean section is not recommended for women with chronic HCV infection alone (216). Hepatitis C acquired in infancy appears to have a benign course, although on biopsy some affected children have chronic hepatitis (210).

Hepatitis G/GB and hepatitis TT

It is unclear whether the hepatitis G/GB virus and the hepatitis TT virus are pathogenic. Both have been shown to be transmitted efficiently from mother to infant, with sustained infection in the offspring, in the absence of any liver disease (217,218,219).

Autoimmune hepatitis

A distinctive clinical characteristic of autoimmune hepatitis is the rapid and complete (or nearly complete) remission that occurs in response to immunosuppression with corticosteroids, given either alone or in combination with azathioprine. The disorder presents frequently in young women, many of whom become anovulatory in response to the active and severe hepatitis, which sometimes progresses to cirrhosis by the time of diagnosis. Women appropriately treated for this disease with corticosteroids and azathioprine regain their fertility, and successful pregnancies without any increase in fatality have been reported (220). The aminotransferase levels may decrease during the second and third trimesters of pregnancy, and some have advocated lowering doses of immunosuppressive drugs during gestation (221). Untreated patients may go into remission during pregnancy (222). The underlying disease may flare up after delivery, and women should be followed up carefully for the first 4 to 6 weeks postpartum, particularly if the immunosuppressive dose has been decreased. Worsening of the underlying disease or even initial presentation of autoimmune hepatitis has been reported during pregnancy (220). Azathioprine has not been reported to be teratogenic in this setting of low-dose therapy. Successful treatment of infertility in these patients, with in vitro fertilization/embryo transfer, has been reported (223).

Primary biliary cirrhosis

The effects of pregnancy on women with primary biliary cirrhosis have been reported to be variable although studies are scarce. Pregnancy may be associated with an increase in cholestasis that resolves after delivery, regression of cholestasis, or progression of disease

including the complications of portal hypertension (224,225,226,227). Ursodeoxycholic acid has been used for ICP with no reports of adverse effects and can be used in patients with primary biliary cirrhosis at the very least during the second and third trimester. In a recent report of nine pregnancies in six women with ursodeoxycholic acid–treated primary biliary cirrhosis, pregnancy was associated with improvement of liver function tests (228).

Wilson disease

Women with ovulatory failure secondary to Wilson disease regain their fertility, often quite rapidly, when treated. Either they or their physicians may be tempted to discontinue therapy during gestation, but, as in nonpregnant patients with Wilson disease, cessation of therapy can have devastating effects and should not be attempted (229,230). Successful pregnancy without teratogenicity has been reported in patients taking penicillamine or trientine (231,232,233,234). Zinc treatment has also been successful during pregnancy (235). Clearly, the major risk to the mother created by stopping therapy greatly outweighs the potential risk to the fetus, and therapy should be continued because successful pregnancy in treated women is the rule (231). Doses of penicillamine or trientine may be reduced during the last trimester (231).

Benign liver tumors (hepatic adenoma, focal nodular hyperplasia, hemangioma)

Hepatic adenoma associated with the previous use of oral contraceptive agents

has been reported to enlarge or rupture during subsequent pregnancy (236,237,238,239), and rupture is associated with high mortality (236). Successful surgical resection of a large hepatocellular adenoma performed at 13 weeks of gestation has been reported; the patient complained of increasing epigastric pain and the tumor (7 cm × 9 cm) was in the left hepatic lobe (240). A recent study of 216 women with focal nodular hyperplasia showed no association between oral contraceptive use and change in lesion size. Twelve women in this group became pregnant, with no change in lesion size or problems with pregnancy (241). An enlargement of a focal nodular hyperplasia associated with the previous use of oral contraceptive was however reported in one pregnant woman (242). The occurrence of liver hemangiomas during pregnancy has been reported (243). The results of a recent prospective study of 94 women (181 hemangiomas) suggests that endogenous and exogenous female sex hormones may play a role in the pathogenesis of liver hemangiomas, although significant enlargement occurs only in a minority of patients (244). These liver hemangiomas are rarely symptomatic and complications are exceptional.

Familial hyperbilirubinemia

The unconjugated hyperbilirubinemia of Gilbert syndrome is not exacerbated by pregnancy (245). In Dubin-Johnson syndrome, however, conjugated hyperbilirubinemia may worsen during gestation but returns to prepregnancy levels after delivery (246). Women with type II Crigler-Najjar syndrome have been reported to have uncomplicated pregnancies during phenobarbital therapy (247,248).

Porphyrias

Porphyrias, genetic disorders of heme metabolism that may be exacerbated by estrogenic hormones, occasionally cause problems for affected women and their fetuses during pregnancy. Porphyria cutanea tarda has rarely been reported to present initially during pregnancy (249). Acute attacks often complicate the course of pregnancy in patients with acute intermittent porphyria, variegate porphyria, or hereditary coproporphyria (250,251), and they may result in intrauterine growth retardation or, rarely, maternal death. On the other hand, many women with acute porphyria, particularly those with little clinical expression of the defect, weather pregnancy with no problems. Precipitation of an initial attack of acute intermittent porphyria by the nausea and vomiting of hyperemesis gravidarum, coupled with antiemetic therapy, has been reported (252).

Liver transplantation

Patients who have undergone transplantation regain their fertility promptly—within weeks of surgery. Young women should be counseled about the risks of becoming pregnant and encouraged to use effective contraception, preferably barrier methods, until their immunosuppressive regimen has been stabilized (253,254). A review of 136 pregnancies after liver transplantation reported to the National Transplantation Pregnancy Registry has shown that most are successful, with no birth defects in the offspring (255). The number of premature infants is increased, and women whose renal function is compromised do not do as well. Ten episodes of acute rejection occurred during pregnancy in this study, and as a result three women lost their grafts. Seven of the women died, three within a year of the pregnancy. The risk of teratogenicity with standard

immunosuppressive agents, including prednisone, azathioprine, cyclosporine, and tacrolimus, is low (256). It is preferable to delay pregnancy until the immunosuppressive regimen has been stabilized (i.e., 1 to 2 years after grafting). Surveillance for infection, such as that with cytomegalovirus, should be increased during gestation. If the results of liver tests become abnormal, then liver biopsy should be performed to establish the nature of the dysfunction. Increased monitoring of the

blood levels of cyclosporine and other immunosuppressive agents is necessary, both before and for several months after delivery (257). Such pregnancies should be considered high risk and managed together with experts in maternal–fetal medicine. Breast-feeding has been discouraged because of concerns about potential immunosuppression of the newborn, but many examples of successful breast-feeding without adverse effects have been reported (255).

Liver transplantation has been accomplished during pregnancy, with and without fetal wastage (255,258,259,260). Livers from living related donors have also been transplanted during pregnancy (261). Obviously, such heroic surgery must be considered with extreme care in this setting. If a condition prompting transplantation is caused (e.g., AFLP) or exacerbated (e.g., Budd-Chiari syndrome) by pregnancy, then prompt diagnosis, followed by termination of the pregnancy with maximum support for the mother, is the treatment of choice. Auxiliary partial orthotopic liver transplantation is an attractive alternative in the setting of liver disease that should resolve after delivery, with regrowth of the patient's native liver and atrophy of the transplanted liver (262).

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Chapter 47
Liver Disease in Infancy and Childhood

Maureen M. Jonas
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Key Concepts

- Understanding the genetic and molecular control of liver development will elucidate hepatobiliary pathophysiology and provide a framework for understanding disorders of the liver and bile ducts that occur at all ages.
- Hepatobiliary disorders that occur in both adults and children, such as viral hepatitis and autoimmune hepatitis, share some features, but there may be important differences in epidemiology, natural history, clinical presentation, and therapeutic considerations.
- Characterization of the genetic defects in several forms of intrahepatic cholestasis illustrates the importance of normal hepatic excretory function, and may explain both severe cholestatic liver disorders in childhood as well as predisposition to liver and biliary tract diseases in some adults.
- Extrahepatic biliary atresia is the most common cause of end-stage liver disease and the most common indication for liver transplantation in the pediatric age-group. Early diagnosis is critical.
- Although some genetic diseases or inborn errors of metabolism cause significant liver disease during infancy and childhood, others may have manifestations beginning in or lasting into adulthood.
- As individuals with cystic fibrosis survive well into adulthood, it has become important to understand the chronic liver disease that develops in some of these patients and devise strategies that may be unique to this large group of patients with a multisystem disorder.

Embryology of the Hepatobiliary System

The hepatic diverticulum is seen as early as the 18th day of gestation (2.5-mm stage) as a thickening of the ventral floor of the distal foregut, the future duodenum. The liver diverticulum penetrates the adjacent mesoderm and capillary plexus known as the *septum transversum*. Cellular interactions between the endoderm and mesoderm result in rapid cell proliferation and the formation of hepatocytes, angioblasts, and sinusoids. By the third and fourth weeks of gestation the growing diverticulum enlarges and branches, projecting into the septum transversum. Division of this diverticulum into a solid cranial portion (hepatic parenchyma) and hollow caudal portion is evident by the 5-mm stage. The hepatic portion differentiates into proliferating cords of hepatocytes and intrahepatic bile ducts while the smaller cystic portion (pars cystica) forms the primordium of the gallbladder, common bile duct, and cystic duct.

The budding liver sequentially invades the vitelline veins and then the umbilical (placental) veins. The vitelline veins run from the gut-yolk sac complex to the heart. The caudal ends of the veins persist as the primitive portal veins and the cranial ends persist as the primitive hepatic veins. The hepatocytes grow as thick epithelial sheets intermingling between branching channels of the vitelline veins within the septum transversum to form a system of connecting liver cell plates,

whereas the proliferative angioblasts become the hepatic sinusoids. The sinusoids, present by 5 weeks' gestation, act as templates for the three-dimensional growth of the hepatic cords. The liver cell plates are initially three to five cells thick. Over time, they gradually transform to one-cell-thick plates, a process that is not complete until 5 years of age. Intrahepatic bile ducts begin to form at 6 weeks' gestation within the hilum of the liver and gradually reach the periphery at 3 months (1).

Although the pars cystica is initially hollow, epithelial proliferation obliterates the lumen early in its development. Therefore, the primitive gallbladder and common bile duct consist of solid chords of epithelial cells directly beneath the developing liver in the 6- to 7-mm embryo. Recanalization of the common bile duct and hepatic duct occurs subsequently, until at the 16-mm stage the proximal gallbladder and cystic duct are hollow. At the third month the gallbladder is fully hollow, and the intrahepatic and extrahepatic biliary structures are joined. Bile secretion into the duodenum starts by the fourth month.

In the third month the liver begins to store iron, and hematopoietic elements derived from the mesenchyme of the septum transversum localize within the lobules and portal tracts. The liver therefore becomes the major blood-forming organ of the embryo. This function is gradually transferred to the developing bone marrow so that by birth only an occasional focus of hematopoiesis remains in the liver.

During fetal life, the falciform ligament conducts the umbilical vein from the umbilicus to the liver. After birth this vein atrophies to form the ligamentum teres. In neonates and infants, the liver accounts for 5% of the total body weight, as compared with 2% in adults.

Development of Hepatobiliary Function

The fetus is supplied with a continuous flow of high-carbohydrate, low-fat, and high–amino acid nutrients through the placenta, but the newborn is fed in intervals with milk; a high fat, lower-carbohydrate diet. At weaning there is another shift to an adult-type diet, which includes more carbohydrates and less fat. The liver plays a central role in these adaptations through regulation of carbohydrate, fat, and protein metabolism. The adult pattern of metabolic pathways develops shortly after birth as the blood supply to the liver changes from one dominated by umbilical venous blood to one in which the hepatic artery plays an equally important role. This allows for the development of the functional zones within the liver, each with unique metabolic demands. Zone 1 (periportal) hepatocytes predominantly perform gluconeogenesis, β-oxidation,

cholesterol biosynthesis, bile acid secretion, ureogenesis, and sulfation of drugs, whereas zone 3 (pericentral) cells perform glycolysis, lipogenesis, ketogenesis, glutamine synthesis, and glucuronidation of drugs.

The liver plays an important role in the handling of dietary starches. Fetal glucose utilization approximately equals umbilical glucose uptake. At weaning, two factors allow for increase in hepatic glucose uptake. First is the presence of a high-capacity, low-affinity glucose transporter, GLUT2, which is insulin-independent. The second is glucokinase, which replaces hexokinase as the predominant glucose phosphorylation enzyme within the hepatocyte allowing for specific action upon glucose, and induction by insulin.

Throughout gestation the fetus actively stores some of the glucose as glycogen so that hepatic glycogen at birth is about twice the adult concentration at 40 to 60 mg/g of liver. Most of this stored glycogen is utilized in the immediate postnatal period. Reaccumulation begins in the second postnatal week, and glycogen stores typically reach adult levels by the third week. The role of this large store is the maintenance of blood glucose levels during the perinatal period, before other energy sources are available, and before the initiation of hepatic gluconeogenesis.

The rate-limiting enzyme involved in gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK), rapidly rises after birth. PEPCK is a cytosolic enzyme primarily expressed in the periportal (zone 1) hepatocytes. Glucose-6-phosphatase catalyzes the final step in glucose release from the liver. This enzyme, located within the lysosomes, rises rapidly at term and is hormonally controlled.

Although the fetal liver is capable of synthesizing albumin, lipoproteins, enzymes, coagulation proteins, and a variety of carrier proteins after the third month of gestation, concentrations of these proteins are low in fetal plasma. Lipoproteins increase in the first week after birth to levels maintained until puberty. Albumin reaches adult levels after several months in a reciprocal relationship with the primary fetal protein, α-fetoprotein. Ceruloplasmin and complement factors increase to mature values during the first year. Transferrin levels are present in the low adult range at birth and slowly rise to normal adult levels thereafter.

Fat storage begins during fetal life. Synthesis of fatty acids by the fetal liver occurs despite low levels of acetyl CoA carboxylase, the rate-limiting step in fatty acid synthesis in the adult liver. The triacylglycerol that accumulated during fetal life is mobilized for local utilization after birth.

Bile acids are produced as early as the tenth week of gestation. Chenodeoxycholic acid is predominant. Although glycine is the most common conjugate in adults, in early life more than 80% of the bile acids are taurine-conjugated. In infants, the total bile acid pool size is small; at 32 weeks' gestation the fetus has a bile

acid pool one sixth that of an adult. In a premature infant, the intraluminal bile acid concentration may fall below the critical micellar concentration of 1 to 2 mmol/L. In addition, there is less effective intestinal reabsorption, inadequate canalicular secretion, and inefficient hepatic uptake of bile acids from the systemic circulation. The cumulative effects of the immature bile acid metabolism and homeostasis in newborns result in relatively inefficient absorption of dietary fats and fat-soluble vitamins, and a tendency toward cholestasis (2).

Hepatocytes are responsible for the excretion of numerous substances through bile, including bilirubin, drug metabolites, and heavy metals such as zinc and copper. Bile secretion starts at the beginning of the fourth month of gestation, and the presence of bile in the lumen of the intestine is responsible for the dark green color of meconium.

Bilirubin formation is 3 to 4 mg/kg per day in healthy adults and 6 to 8 mg/kg per day in healthy term infants. This difference is due to the greater relative red blood cell mass and shorter red blood cell life span in infants. Infants have lower levels of the conjugating enzyme, bilirubin glucuronyl transferase, and therefore have fewer diglucuronides than adults. When bilirubin conjugates enter the intestinal lumen, normal bacterial flora hydrogenate the carbon double bonds to produce urobilinogens, which are excreted. Neonates lack the bacteria *Clostridium ramosum* and *Escherichia coli* and are therefore more likely to absorb bilirubin from the intestine. Bilirubin can also be unconjugated by bacterial or tissue β-glucuronidase, and readily absorbed from the intestine.

Congenital Abnormalities in Hepatobiliary Structure

Situs Inversus and Heterotaxia

Situs inversus and heterotaxia result in left sided or ambiguous location of the liver within the abdominal cavity, respectively. Either may occur with other anomalies such as in the polysplenia/asplenia syndromes.

Vascular Anomalies

Although many variations have been described in hepatic artery anatomy, most do not have clinical significance except when the patient requires hepatic surgery.

Congenital absence of the portal vein (CAPV) is a rare but well-described anomaly (2a), and when seen with a spontaneously occurring mesocaval or other portosystemic shunt, is referred to as Abernethy malformation. CAPV may be associated with nodular regenerative hyperplasia of the liver (2) and the Goldenhar's syndrome (3). Children with this anomaly may have elevated serum ammonia, bile acids, and galactose concentrations, as the portosystemic shunt bypasses the detoxifying ability of the liver (4). Other anomalies of the portal vein are occasionally seen, in association with cardiac or urinary tract abnormalities.

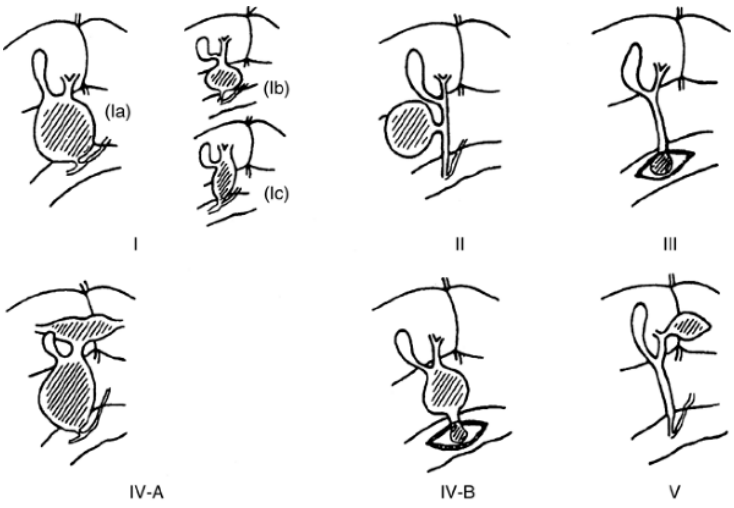
The presence of multiple serpiginous collateral veins surrounding a small or thrombosed portal vein has been referred to as *cavernous transformation of the portal vein*. This is an acquired rather than a congenital abnormality and is accompanied by portal hypertension since portal resistance is markedly elevated. It represents the body's effort to maintain hepatopetal portal flow in the face of occlusion of the extrahepatic portal vein. The causes of portal vein thrombosis include umbilical infection (omphalitis), perinatal catheterization of the umbilical vein, pancreatitis, surgical manipulation during splenectomy, and hypercoagulable states including deficiencies of protein C, protein S, or antithrombin III, and the presence of anticardiolipin antibodies or a factor V Leiden gene mutation. Children with cavernous transformation of the portal vein typically come to medical attention within the first decade with splenomegaly or bleeding from esophageal varices. The diagnosis is confirmed by ultrasonography of the extrahepatic portal area with Doppler interrogation. Endoscopic ligation or sclerosis of esophageal varices, portosystemic shunts, and mesenterico-left portal bypass (Rex shunt) are palliative measures. The natural history is such that over time there is a decrease in the frequency and intensity of the hemorrhagic manifestations. Liver histology is usually nondiagnostic with nonspecific findings, which may include fibrosis.

Biliary Tract Abnormalities

Choledochal cysts

Choledochal cysts are congenital malformations resulting in cystic dilatation of part or all of the extrahepatic biliary system. The incidence is estimated at 1 in 13,000 to 200,000 live births. They are found in women four times more frequently than in men, and are more prevalent in Asians, specifically the Japanese. The location of the cyst allows for classification into one of five anatomic types as described by Todani (Fig. 47.1) (5). Most are type I, diffuse enlargement of the common bile duct. The etiology of cyst formation is unclear, although there is growing evidence that the dilatation results from an anomalous junction of the common bile duct and the pancreatic duct resulting in a common channel up to 3.5 cm in length, from a normal of 5 mm. This long common channel may allow for the reflux of pancreatic proteases into the extrahepatic biliary tree resulting in cholangitis and stenosis. This hypothesis is supported by high levels of amylase within the cysts, but

the frequent documentation of prenatal choledochal cysts makes a malformation etiology more likely.



▪ **Figure 47.1** Types of choledochal cyst. (From Todani T, Watanabe Y, Narusue M, et al. Congenital bile duct cysts: Classification, operative procedures and review of thirty seven cases including cancer arising from choledochal cyst. *Am J Surg* 1977;134:263–269.with permission.)

Most patients present within the first decade of life. The classic triad of abdominal pain, jaundice, and palpable right upper quadrant mass occurs in less than 20% of patients. Other common symptoms are fever, nausea, and vomiting, with or without associated pancreatitis. Ultrasonography is the most valuable diagnostic test. Radionuclide scanning may demonstrate the accumulation of tracer within the cyst. Endoscopic retrograde cholangiopancreatography (ERCP) provides delineation of the biliary anatomy, but is not always necessary to confirm the diagnosis.

The high incidence of biliary malignancy, reported between 2.5% and 17.5% in Japanese patients, has led to the recommendation of cyst excision. Most tumors are adenocarcinomas, detected at a mean age of 35 years. The cause of the malignant transformation is not known, but may relate to the chronic reflux of pancreatic proteases and the mutagenic potential of secondary bile acids in a stagnant environment. The reconstruction varies on the cyst type, anatomy, and surgical preference; the most common procedures are hepaticoduodenostomy, hepaticojejunostomy, or jejunal interposition.

Gallbladder anomalies

Congenital absence of the gallbladder occurs in 1 in 7,500 to 1 in 10,000 individuals. Failure of development of the pars cystica is the likely etiology. As an isolated abnormality this is of little clinical significance although rarely symptoms develop because of calculi in the ductal system. In addition to extrahepatic biliary atresia (EBA), which may accompany agenesis of the gallbladder, other associations include imperforate anus, genitourinary anomalies, anencephaly, bicuspid aortic valve, and cerebral aneurysms. *Hypoplasia of the gallbladder* has been described. As many as one third of individuals with cystic fibrosis (CF) have small, poorly functioning gallbladders. There is also an association with EBA and trisomy 18. The incidence of *double gallbladder* is 0.1 to 0.75 per 1,000. The two cystic ducts may converge into a single duct forming a Y-shaped structure, or an accessory gallbladder may lie under the left lobe of the liver, draining into the left hepatic duct.

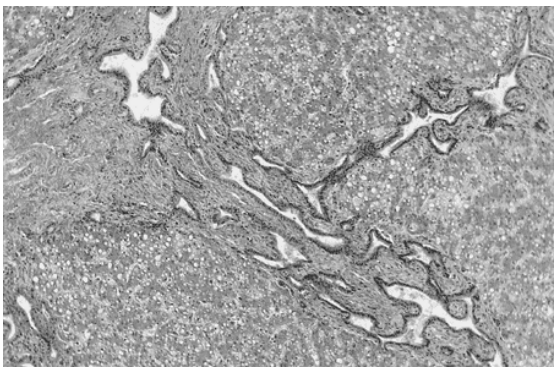
Extrahepatic biliary atresia—fetal form

EBA is usually considered an acquired condition, and is discussed in subsequent text in this chapter. However, there is an embryonic or fetal type that comprises approximately 35% of cases and may be a true congenital biliary tract anomaly (6). This form is characterized by earlier onset of cholestasis and absence of bile duct remnants. Ten percent to 20% of children with fetal-type biliary atresia have associated anomalies. The most common are various combinations that characterize the laterality sequence, such as polysplenia or asplenia, cardiovascular defects, abdominal situs inversus, intestinal malrotation, and vascular aberrations of the portal vein and hepatic artery. Intestinal malrotation alone is seen in 12%. There is speculation that the fetal form of biliary atresia has a different pathologic mechanism than the perinatal type, although this has not been proven.

Ductal plate abnormalities

At about the eighth week of gestation, the hepatic precursor cells that lie adjacent to the hilar portal vein vessels form a sleeve-like double layer of cells, which extends toward the periphery along the intrahepatic portal vein branches. This structure has been called the *ductal plate* (DP). Beginning at 12 weeks' gestation and extending into the postnatal period, the DP undergoes remodeling (7). Individual bile ductules are incorporated into the periportal mesenchyme that surrounds the portal vein branches. During successive periods of fetal life, DP remodeling leads to the formation of the intrahepatic biliary tree. The largest ducts are formed first, followed by segmental, interlobular, and finally the smallest bile ducts. Arrest or derangement in remodeling leads to the persistence of primitive bile duct configurations, or to what Jorgensen termed *ductal plate malformation* (DPM) (7). The occurrence of DPM at different generation levels of the developing biliary tree gives rise to different clinicopathologic entities, such as congenital hepatic fibrosis (CHF) and Caroli syndrome (8,9).

The most common DPM-associated disorder is autosomal recessive polycystic kidney disease (ARPKD) with CHF, which occurs with an incidence between 1 in 6,000 and 1 in 40,000 births. The cysts are rarely macroscopically visible. Microscopically, the portal tracts appear enlarged by connective tissue and contain tortuous and dilated bile duct structures (Fig. 47.2) and incompletely remodeled ductal plates, which are in continuity with the rest of the biliary system. The renal lesion is characterized by radially arranged tubular collecting duct cysts occupying most of the large, externally smooth renal mass. This disorder has been attributed to mutations in *PKHD1* on chromosome 6p12, which encodes a protein called *fibrocystin*. Fibrocystin is localized to the collecting ducts and biliary ducts.



▪ **Figure 47.2** Congenital hepatic fibrosis. Expanded portal tracts show persistence of the ductal plate and cystic and tortuous bile ducts. Incidental mild steatosis is also present.

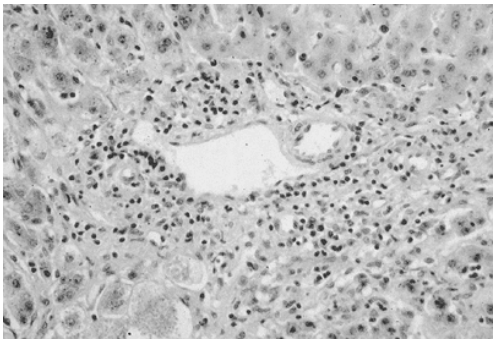
CHF may also be associated with other liver malformations such as von Meyenburg complexes (bile duct microhamartomas), as well as other renal lesions including autosomal dominant polycystic kidney disease, renal dysplasia, and nephronophthisis. Congenital dilatation resulting from DPM of the larger, segmental intrahepatic bile ducts is termed *Caroli disease*. When this lesion is combined with the changes of CHF, as is typically the case, the disorder is termed *Caroli syndrome* (see Chapter 43). Mutations in *PKHD1* have been found in 32% of adults with CHF/Caroli syndrome (10).

Infants with congenital hepatic fibrosis–autosomal recessive polycystic kidney disease (CHF-ARPKD) may have enlarged and severely dysfunctional kidneys. In this setting, hepatic fibrosis may be present, but is rarely an important clinical factor. In older patients, the most significant abnormality is portal hypertension due to the hepatic fibrosis and/or portal vein anomalies, such as duplication of intrahepatic branches. Hematemesis or melena may occur as early as 1 year, but more typically at 5 to 13 years of age. The biochemical parameters of hepatic synthetic function are typically normal, but there is a risk of cholangitis. Treatment may include portosystemic shunting for portal hypertension and aggressive antibiotic therapy for cholangitis. Approaches such as variceal sclerotherapy or pharmacologic management of portal hypertension have been used. In patients with chronic cholangitis and/or progressive hepatic dysfunction, liver transplantation may be indicated.

Bile duct paucity syndromes

Paucity of the intrahepatic bile ducts is defined as a ratio of interlobular ducts to

portal tracts of less than 0.9 (Fig. 47.3). Surgical biopsy or serial needle biopsies may be required to examine at least 20 portal tracts, the recommended sample for this diagnosis, but as few as 5 portal tracts in a percutaneous biopsy may be sufficient in the appropriate clinical setting. Syndromic paucity of the bile ducts, or Alagille syndrome (AS), discussed in subsequent text, is the most common disorder with this finding. Patients with nonsyndromic paucity of intrahepatic bile ducts typically present earlier than those with AS (11). Paucity of the bile ducts has been described in association with a variety of other conditions including Down's syndrome, hypopituitarism, CF, α_1-antitrypsin deficiency, Zellweger's syndrome, Ivemark's syndrome, and congenital infections. Inflammatory destruction of bile ducts occurs in graft versus host disease, chronic hepatic allograft rejection, primary sclerosing cholangitis (PSC), and drug toxicity (vanishing bile duct syndrome). Paucity of the bile ducts may lead to chronic cholestatic liver disease with biliary-type cirrhosis.



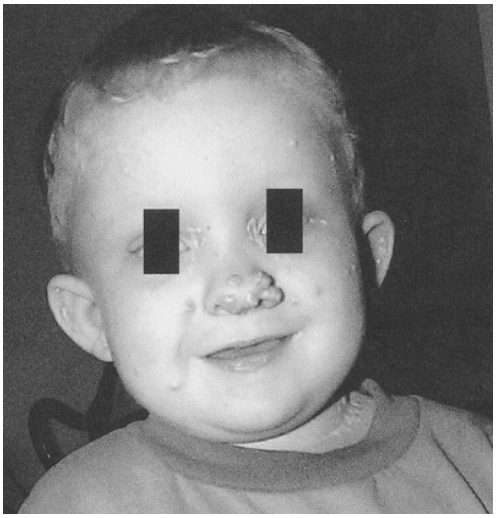
▪ **Figure 47.3** Intrahepatic paucity of bile ducts. Portal tract with absent bile duct in a patient with Alagille syndrome.

AS is also called *arteriohepatic dysplasia*, and is characterized by reduced interlobular bile ducts in association with cardiac, skeletal, ocular, facial, and less frequently renal, and neurodevelopmental abnormalities (12). Features of AS are listed in Table 47.1 (12,13). The prevalence of this autosomal dominant syndrome with highly variable penetrance is reported as 1 in 100,000 live births. Histologic evidence of bile duct paucity may not be present at birth; the ducts are thought to be lost over a number of months or even years. Jaundice is present in most symptomatic patients and presents as conjugated hyperbilirubinemia (serum levels between 4 and 10 mg/dL) in the neonatal period. Hepatomegaly is invariably present and pruritus can become severe by 6 months. Xanthomas develop in patients with chronic hypercholesterolemia and can become disseminated and disfiguring (Fig. 47.4). General management includes nutritional support with provision of fat-soluble vitamin supplements and symptomatic treatment for pruritus. Some of these symptoms may be improved by partial external biliary diversion (14). In some cases, the cardiac manifestations dominate the clinical presentation, and corrective surgery for peripheral pulmonic stenosis or other lesions may be needed. Progression to liver failure and cirrhosis requiring liver transplantation occurs in approximately 15% of cases, and account for approximately 2% of pediatric liver transplantations. In other cases, the cholestasis improves as the child approaches adulthood. Vascular anomalies, such as aneurysms and coarctation of the aorta, are reported in 9% of patients, and are responsible for up to 34% of the mortality caused by this syndrome (15). The genetic defect of AS has been found on chromosome 20p12, with a deletion or mutation of a single copy of the *Jagged 1* gene (16). Alterations of this gene interrupt the Notch signaling pathway that is crucial for cell-to-cell communication during differentiation. Notch signaling has an important role in the differentiation of biliary epithelial cells and is essential for their tubular formation during intrahepatic bile duct development (17).

Table 47.1. Features of Alagille Syndrome (12,13)

Feature	Percentage of patients
Bile duct paucity	85–100
Chronic cholestasis	91–96
Peripheral pulmonic stenosis	67–70
Tetralogy of Fallot	9–14
Butterfly vertebrae	51–87
Characteristic facies[a]	95–96
Posterior embryotoxon[b]	78–88
Growth retardation	50–87

[a]Facies consist of prominent forehead, moderate hypertelorism with deepset eyes, small pointed chin, saddle or straight nose.
[b]A defect in the anterior chamber of the eye in which there is a prominence of Schwalbe's ring (this is seen in 10% of the normal population).
Data from Alagille D, Estrada A, Hadchouel M, et al. Syndromic paucity of interlobular bile ducts (Alagille syndrome or arteriohepatic dysplasia): review of 80 cases. *J Pediatr* 1987;110:195–200 and Emerick K, Rand EB, Goldmuntz E, et al. Features of Alagille syndrome in 92 patients: frequency and relation to prognosis. *Hepatology* 1999;29:822–829.



• **Figure 47.4** Cutaneous xanthomas. The skin of this child with Alagille syndrome has numerous xanthomas that may be plaques or nodules with characteristic smooth surface. The lesions are characteristically yellow or pink.

Genetic/Metabolic Liver Disease

The liver is involved primarily or secondarily in many inborn errors of metabolism. This section will focus on those disorders that lead to acute or chronic

damage to the liver itself by one of three mechanisms: accumulation of toxic metabolites, failure to produce essential compounds, or sequestration of an abnormally synthesized product within the liver. In addition to nonspecific signs of liver disease, such as those seen in viral hepatitis or drug-induced liver injury, metabolic liver disease is often associated with other signs and symptoms that should suggest this diagnosis to the clinician (Table 47.2). Adequate liver sampling allows measurement of enzymatic pathways or substrate accumulation in addition to histologic examination. A specific diagnosis may allow effective therapy or provide an indication for liver transplantation. Genetic counseling is often indicated.

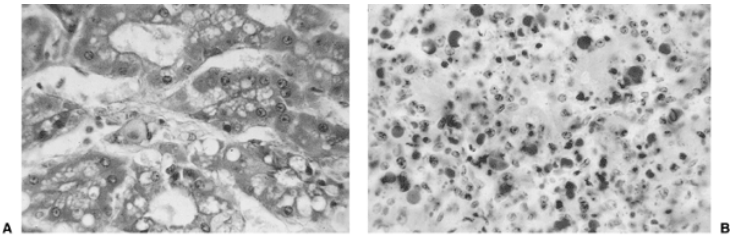
Table 47.2. Clinical Features Suggestive of Metabolic Liver Disease in Children

Coma with hyperammonemia
Hypoglycemia
Psychomotor retardation
Hepatosplenomegaly with or without liver dysfunction
Acidosis
Failure to thrive
Muscle weakness
Coagulopathy, particularly out of proportion to liver test abnormalities
Dysmorphic facial features
Cholestasis
Cardiac disease

Disorders of Carbohydrate Metabolism

Galactosemia

Galactose is normally converted to glucose through three separate enzymatic reactions involving galactokinase, galactose 1-phosphate uridyl transferase (GALT), and uridine diphosphate galactose 4-epimerase. The three known disorders are now designated transferase deficiency galactosemia, epimerase deficiency galactosemia, and galactokinase deficiency galactosemia. The overall frequency is approximately 1 in 40,000 live births. The most common severe defect involves a deficiency of GALT. The sequence of the human *GALT* gene has been established (18). Many patients are found to be compound heterozygotes. Inactivity of the transferase results in the accumulation of toxic metabolites including galactose 1-phosphate and galactitol. Early manifestations include lethargy, vomiting, acidosis, cataracts, failure to thrive, and jaundice. Urinary tract infection and/or sepsis, typically with gram-negative species, is also a common presenting problem. Hemolytic anemia and erythroid hyperplasia, occasionally severe and resembling erythroblastosis, occur in 40% of patients. Rapid recognition and treatment in the newborn period is critical because untreated disease is likely to result in severe neurologic injury. Most states have programs for newborn screening for galactosemia, but the diagnosis is confirmed with an enzymatic assay for GALT using red blood cells. Histopathologic findings in the liver include diffuse hepatocellular damage with marked steatosis, cholestasis, and pseudoacinar transformation (Fig. 47.5A,B). Treatment consists of strict elimination of galactose from the diet, which will normally reverse the hepatopathy. However, the long-term efficacy of dietary therapy may not be as successful due to difficulty in completely eliminating galactose from the diet and from endogenous conversion of glucose into galactose through reversal of the normal pathways for galactose metabolism. Long-term complications include growth failure, developmental delay, and ovarian failure despite vigilant adherence to a galactose-free diet (19).



▪ **Figure 47.5** Galactosemia. **A:** Pseudoacinar transformation of hepatocytes, fatty change and cholestasis is the usual triad of histologic findings. **B:** Oil-red-O on a frozen section reveals the extensive hepatocellular steatosis.

Hereditary fructose intolerance

Hereditary fructose intolerance (HFI) is the result of a genetic deficiency in the enzyme fructose 1,6-biphosphate aldolase (aldolase B). The pathophysiologic process is due to metabolic effects of the

accumulated fructose 1-phosphate. Sorbitol is interconverted to fructose and can therefore lead to a similar process. Signs and symptoms of long-term exposure to fructose include hepatomegaly, abnormal aminotransferase values, hepatic steatosis, poor feeding, vomiting, irritability, and poor growth (20). The classic features of acute metabolic disease and hypoglycemia are not always present, because many affected individuals evolve an eating behavior that avoids fructose. Therefore, HFI needs to be considered in children with unexplained hepatomegaly and steatosis. A high index of suspicion is crucial because the presenting signs and symptoms can be subtle. The histologic findings are similar to those of galactosemia. Aldolase B activity can be measured in liver tissue. Three different specific mutations in aldolase B account for a large number of affected individuals. Deoxyribonucleic acid (DNA) diagnostic assays (including allele specific oligonucleotide hybridization) using peripheral leukocytes may be utilized. Treatment involves strict avoidance of fructose, sorbitol, and sucrose. Partial adherence to this difficult diet can ameliorate many of the acute manifestations of this disease but not the chronic problems such as growth failure.

Table 47.3. Glycogen Storage Diseases

Type	Enzyme deficiency	Tissue involved	Synonym/notes
0	Glycogen synthetase	Liver, muscle	Aglycogenosis
Ia	Glucose-6-phosphatase (G6Pase)	Liver, kidney, intestine	Von Gierke's disease (hepatorenal glycogenosis)
Ib	Translocase (T_1) responsible for movement of glucose-6-phosphate across intracellular membranes	G6Pase activity is normal in homogenate made of frozen liver but is deficient in isotonic homogenate made of fresh (unfrozen) liver	
Ic	Translocase (T_2, the microsomal phosphate/pyrophosphate transport protein)		
Id	Translocase T_3		
IIa infantile IIb adult	Lysosomal acid α-glucosidase	In the fatal, classic form (IIa), glycogen concentration excessive in all organs examined; enzyme deficiency may be generalized; cardiac muscle in IIb normal but deficient in α-glucosidase activity	Pompe disease (generalized glycogenosis, cardiac glycogenosis)
III	Amylo-1,6-glucosidase ("debrancher" enzyme)	Liver, muscle, heart, etc., in various combinations	Limit dextrinosis (debrancher glycogenosis); Cori disease; Forbes disease

IIIa		Liver only	
IIIb		Generalized	
IV	Amylo-1,4→1,6-transglucosidase ("brancher enzyme")	Generalized (?)	Amylopectinosis (brancher glycogenosis); Andersen disease
V	Muscle phosphorylase deficiency	Skeletal muscles only	McArdle syndrome (liver and smooth muscle phosphorylase not affected)
VI	Liver phosphorylase deficiency	Liver; skeletal muscle normal	Hers disease
VII	Phosphofructokinase	Skeletal muscle, erythrocytes	Tarui disease
VIII	Hepatic phosphorylase kinase	Liver, brain, skeletal muscle normal; cerebral glycogen increased	Liver glycogenesis, X-linked
IX	Liver phosphorylase-*b*-kinase deficiency	Liver only; muscle tissue normal biochemically and microscopically	

Glycogen storage diseases

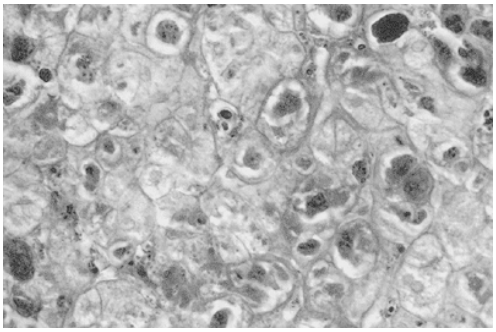
Glycogen, a polysaccharide molecule composed of D-glucose units, is found predominantly in liver and muscle. The glucose units are joined linearly by 1,4 linkages with 1,6 linkage branching at every fourth unit. The glycogen storage diseases (GSD) are disorders characterized by accumulation of glycogen in organ-specific patterns based on the deficient enzyme (Table 47.3).

Clinical manifestations of the various GSDs may result from inability to utilize glycogen stores, accumulation of glycogen within the liver and/or other tissues, and the toxic effects of certain abnormal types of glycogen. Forms of GSD that primarily affect the liver produce diffuse hepatomegaly and hypoglycemia, which

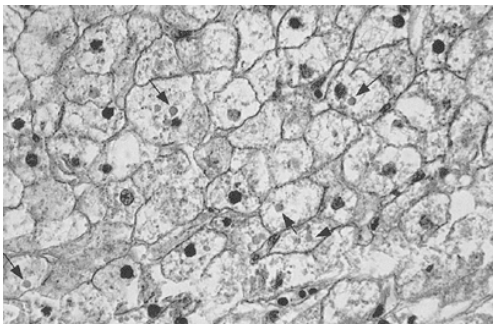
may be life-threatening. Fasting hypoglycemia is the hallmark of GSD due to an inability to utilize glycogen stores to produce glucose; type I is the most common. Forms of type I GSD (Table 47.3) include deficiency of glucose 6-phosphatase (von Gierke's disease, type Ia) and various deficiencies in glucose and glucose metabolite transport (glucose 6-phosphate transport, type Ib, microsomal phosphate transport, type Ic, microsomal glucose transport, type Id). GSD type Ib presents in a clinical manner identical to GSD type I, but these patients are often neutropenic with impaired neutrophil function (21); they are prone to recurrent bacterial infection, oral and intestinal mucosal ulceration, and bleeding. The striking limitation of glucose transport across the neutrophil cell membrane may account for impairment of neutrophil function. Cytokine therapy may correct the neutropenia.

A very different clinical presentation is observed in those disorders that lead to hepatic accumulation of toxic forms of glycogen. The best example of this is GSD type IV, deficiency of the glycogen branching enzyme. Glycogen that accumulates in this disease has long chains of glucose in a 1,4 linkage, resembling plant starch or amylopectin. This form of glycogen is relatively insoluble and presumed to be toxic. Liver biopsy reveals characteristic intracytoplasmic masses of periodic acid-Schiff (PAS) positive, diastase resistant material that enlarge and distort hepatocytes (Fig. 47.6). Progressive liver disease therefore becomes a major distinguishing feature of GSD type IV. Portal hypertension and hepatic failure may develop in early childhood.

Specific diagnosis depends on demonstration of the deficiency of enzyme activity in biopsy specimens. In these disorders, the liver biopsy should be processed for light and electron microscopic evaluation and a portion rapidly frozen for subsequent biochemical analysis. The light microscopic appearance of the liver in type I, the most common variety, demonstrates abundant accumulation of free glycogen in the cytoplasm and mild steatosis. Hepatocytes are markedly enlarged with clear cytoplasm, central nuclei, and compression of adjacent sinusoids (Fig. 47.7). Glycogenated nuclei are numerous in zone 1. Diagnosis of the specific type of GSD is critical for proper treatment and prediction of prognosis and potential complications. Specific enzymatic assays and DNA diagnostic tests are available in specialty laboratories for each of the disorders. Diagnostic assays can be performed on a number of tissues including liver, muscle, leukocytes, and fibroblasts.



▪ **Figure 47.6** Glycogenosis type IV (amylopectinosis). Large intracytoplasmic PAS+ diastase resistant inclusions enlarge and deform hepatocytes.



▪ **Figure 47.7** Glycogenosis type I. Diffusely enlarged hepatocytes have clear cytoplasms that impart a plant-like appearance. Hepatocellular megamitochondria (*arrows*) are usually present.

GSD can present at any age, and the prognosis is highly variable. Early reports of correction of many of the metabolic derangements of GSD type I by provision of nutrients directly into the systemic circulation by total parenteral nutrition (TPN) or by portacaval shunting gave important clues to its pathogenesis and therapy. Subsequently, it was demonstrated that maintenance of blood glucose levels greater than 70 to 90 mg/dL obviated most of the clinical manifestations of GSD type I (22). The presumed mechanism of action for this therapy is reduction of the stimulus for glycogenolysis and prevention of the abnormal compensatory metabolic pathways leading to lactate accumulation, lipid synthesis, purine synthesis, and subsequent hyperuricemia. The neutropenia of GSD type Ib is not corrected by glucose homeostasis.

Originally, maintenance of blood glucose was accomplished by continuous enteral feeding of infants with nasogastric or gastrostomy tubes. Alternatively, it has been shown that frequent daytime feedings (every 2 to 3 hours) of a high-starch diet can be combined with continuous nighttime tube feedings with the same effects. Frequent feeding of high-carbohydrate–containing foods and nocturnal administration of slow-release glucose polymers, such as uncooked cornstarch are utilized (22,23). This prevents the development of hypoglycemia and also

limits incorporation of excess dietary glucose into glycogen. However, as children become older, this regimen is more difficult to maintain and is altered to include daytime meals supplemented with raw cornstarch. Cornstarch undergoes slow degradation to glucose by α-amylase and, when given every 6 hours, steadily releases sufficient glucose into the system. This cornstarch supplementation can be combined with continuous nighttime feedings until the children are through the period of rapid growth and their glucose requirement decreases to the point that cornstarch supplements alone are sufficient for metabolic control.

As patients with GSD type I approach late adolescence, the tendency for hypoglycemia decreases and some investigators believe that less rigorous dietary therapy is required. However, treatment of the secondary complications of the disease with agents such as allopurinol for hyperuricemia or lipid-lowering agents for the prevention of cardiovascular disease and pancreatitis may be necessary. With prolonged survival due to improved therapy, an increasing number of patients are surviving into adulthood; this has led to recognition of late complications such as altered bone mineralization, renal disease, and endocrine abnormalities (24).

A long-term complication of GSD type I for which no definitive therapy has been established is hepatic adenoma (25,26). It is believed that adenomas develop because of chronic stimulation of the liver by glucagon and other trophic agents produced in response to chronic or recurrent hypoglycemia. Although some authors have reported regression of adenomas with aggressive dietary therapy, many patients who have received this treatment since infancy or early childhood are just now reaching adulthood and adenomas are a common finding in this group. Currently, a major challenge in the management of patients with GSD type I is the prevention, detection, and management of these lesions, as well as the development of a strategy for early detection of the rare transformation of adenomas into malignant lesions. In addition, a link between the hepatic adenomas and the normocytic anemia that often accompanies this disorder has been made. Hepcidin was found in abundance in the adenomas in GSD type Ia, and resection of the adenomas has resulted in normalization of the hematologic parameters and iron studies (27). Guidelines regarding monitoring of adenomas have been published, suggesting abdominal ultrasonography and measurement of serum α-fetoprotein and carcinoembryonic antigen every 3 months, as well as

computed tomography (CT) scan or magnetic resonance imaging in the case of growth or blurring of the margins of the lesions (28). Liver transplantation has been performed in patients having GSD, with some success (29). It is important to remember that these are systemic diseases that may have variable degrees of involvement of both skeletal and cardiac muscles.

Carbohydrate-deficient glycoprotein syndromes (glycosylation disorders)

The carbohydrate-deficient glycoprotein (CDG) syndromes are a group of newly recognized inborn errors of glycoprotein metabolism characterized by abnormal synthesis of *N*-linked oligosaccharides (30). The biochemical hallmark is a partial deficiency of the carbohydrate moiety of a wide range of secretory glycoproteins, including binding proteins, lysosomal enzymes, and coagulation factors. The clinical manifestations of the CDG syndromes, which include significant liver disease, are the direct embryologic and physiologic consequences of the abnormal *N*-linked glycosylation of cell structures.

CDG syndrome type I is a genetic multisystem disorder (31). The clinical picture is dominated by nervous system dysfunction, resulting in psychomotor retardation, seizures, ataxia, and stroke-like episodes (due to hypercoagulability). Also noted are an abnormal pattern of subcutaneous fat distribution (lipodystrophy and inverted nipples), feeding difficulties, retinitis pigmentosa, hypoalbuminemia/protein-losing enteropathy, pericardial effusion, and/or ascites. There is an age-dependent constellation of abnormalities in gonadal, thyroid and growth hormone, and insulin. Light microscopic findings in liver biopsy specimens include fibrosis and occasional cirrhosis and steatosis (32).

The CDG syndrome type I is clinically and biochemically distinct from type II, which is due to a deficient activity of the Golgi enzyme *N*-acetylglucosaminyltransferase II, and from other CDGs. The primary defect in CDG-I is the subject of ongoing study, although phosphomannomutase (PMM) deficiency appears to be the major cause (30). Biochemical diagnosis is based on documentation of misglycosylation, usually through detection of altered isoelectric forms of serum glycoproteins. The biochemical changes are easily observed by detecting altered isoelectric focusing of serum transferrin (carbohydrate-deficient transferrin). Serum antithrombin III and thyroxine-binding globulin (TBG) levels are low; these are useful screening probes for this disorder. Prenatal diagnosis is possible because PMM is active in amniocytes. No known treatment is available. Levels of intracellular mannose are limited and exogenous mannose can correct the defect in protein glycosylation in vitro; therefore some patients may benefit from including oral mannose in their regular diets.

Disorders of Lipid Metabolism

Wolman's disease and cholesteryl ester storage disease

Two distinct clinical syndromes, Wolman's disease and cholesterol ester storage disease (CESD), are

characterized by the massive intralysosomal accumulation of lipid (triacylglycerols and cholesteryl esters). The severe infantile-onset Wolman's disease and the milder late-onset CESD are caused by mutations in different parts of the lysosomal acid lipase gene, which is located on chromosome 10q24-q25. The diagnosis of these allelic disorders is made by demonstration of deficient lysosomal acid lipase activity in leukocytes or fibroblasts and an elevation of triglycerides and cholesterol ester levels in tissue. There is no specific therapy for these two disorders.

Wolman's disease (33) is characterized clinically by steatorrhea, failure to thrive, hepatosplenomegaly, and jaundice; the disease causes death usually within the first year of life. Stippled calcification of the adrenal glands is uniformly present either early in the course or in association with terminal liver failure. The disease has been reported in several ethnic groups and appears to be inherited as an autosomal recessive trait. There is diffuse cellular accumulation of triacylglycerol and cholesterol ester in lymph nodes, bone marrow, small intestine, liver, spleen, and adrenal glands. Deficiency of lysosomal acid lipase activity directed toward the hydrolysis of either triglyceride or cholesterol ester has been demonstrated. Grossly, the liver is yellow; frozen sections show lipid droplets in the parenchymal cells, some of which contain birefringent crystals under polarized light. Routinely processed liver biopsy reveals macrovesicular and microvesicular steatosis with empty crystalline profiles. Plasma lipids are generally normal. Diagnosis depends on liver biopsy and study of frozen sections with polarized light in addition to routine staining, analysis of tissue lipid, and demonstration of acid lipase deficiency. Death has occurred in all cases despite therapeutic trials of cholestyramine, adrenal steroids, clofibrate, cyclophosphamide, antibiotics, and thyroxine.

In patients with CESD, cholesteryl ester and triglyceride also accumulate in the liver and the intestinal mucosa; there is a marked decrease in lysosomal acid lipase activity against these substrates. The diagnosis can be confirmed by documenting deficiency of lysosomal acid cholesteryl hydrolase activity in fibroblasts (34). Although biochemical abnormalities are similar to those found in patients with Wolman's disease, patients with CESD have a normal life expectancy and present with hepatomegaly and hyperbetalipoproteinemia only, possibly due to a higher residual enzyme activity. Hepatomegaly is present at birth and increases with age. Grossly, the liver is similar in appearance to that in Wolman's disease, a brilliant orange-yellow color. Histologic findings on frozen section and routine light microscopy are also similar. By electron microscopy (EM), the larger vacuoles are seen to be surrounded by a single membrane, to contain angular images of cholesterol crystals, and to be secondary lysosomes. The epithelial cells of the intestinal mucosa are normal, but the lamina propria contains crystals of cholesterol ester and masses of foam cells around the lacteals. Suppression of cholesterol synthesis and apolipoprotein B production by 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in combination with a diet excluding foods rich in cholesterol and triglycerides results in clinical improvement in some cases of CESD.

Gaucher's disease

Gaucher's disease, due to deficiency of acid β-glucosidase, is associated with widespread accumulation of glucosyl-ceramide laden macrophages, resulting in hepatosplenomegaly, marrow replacement causing skeletal disease, lung infiltration, and sometimes neurologic disease. There are several types, with different ethnic and geographic patterns. Hepatomegaly is seen in more than 50% of patients, but cirrhosis and portal hypertension are uncommon. Liver histology demonstrates the glycolipid-filled macrophages (Gaucher's cells) in the sinusoids, but hepatocytes are spared (35). This is probably due to biliary excretion of the glucocerebroside and the handling of glycolipids by mononuclear phagocytes, rather than hepatocytes. The diagnosis is confirmed by measurement of acid β-glucosidase in peripheral blood leukocytes. Enzyme replacement therapy is available, but responsiveness is variable. If cirrhosis is present, response to enzyme therapy is typically poor.

Niemann-Pick disease

The group of disorders classified under the eponym of Niemann-Pick disease (NPD) has been divided into two broad types: (a) A and B NPD are lysosomal storage disorders that result from deficient activity of acid sphingomyelinase (ASM); and (b) type C NPD is a lipidosis distinguished by a unique error in cellular trafficking of exogenous cholesterol. Both types are characterized by varying degrees of hepatomegaly, foam cells in the liver, bone marrow, and other tissues, and variably increased amounts of sphingomyelin, cholesterol, glycosphingolipids, and bis (monoacylglycero)-phosphate in the visceral organs.

Hereditary Tyrosinemia Type I

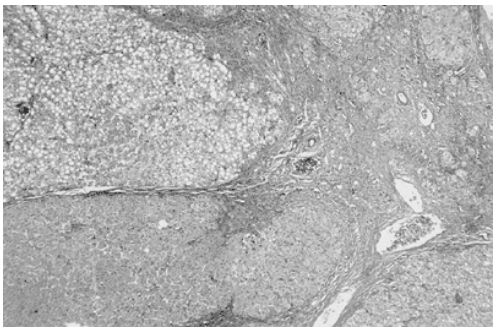
Transient neonatal hypertyrosinemia is a self-limiting condition primarily of premature infants, probably caused by an immaturity in tyrosine aminotransferase activity. Hypertyrosinemia also occurs with any severe hepatic injury, usually in association with high serum methionine levels. Hereditary tyrosinemia (HT) type I is an autosomal recessive disorder caused by deficiency of fumarylacetoacetate hydrolase (FAH), the last enzyme in the tyrosine degradation pathway. The

FAH gene has been mapped to chromosome 15 at q23-q25 (36) and cloned; more than 25 mutations have been associated with the phenotype of HT. Autosomal recessive inheritance has been suggested. The worldwide incidence is approximately 1 in 100,000 births. Because of a complex founder effect, there is an unusually high prevalence (1 in 1846) of HT in the province of Quebec, Canada, particularly in the Saguenay-Lac Saint Jean region.

Metabolites that accumulate proximal to the enzymatic block such as succinyl acetate, succinyl acetone, fumaryl acetoacetate, and maleylacetoacetate are highly reactive electrophilic toxic compounds, which bind to sulfhydryl groups, often leading to tissue injury. Many secondary enzymatic and biochemical defects occur in tyrosinemia because of the accumulation of these precursor compounds. For example, succinyl acetoacetate can inhibit enzymes such as porphobilinogen synthase, leading to the accumulation of 5-aminolevulinic acid and symptoms of acute intermittent porphyria.

There are two forms of HT, acute and chronic, which may occur in the same family. The acute form presents in infancy with severe liver dysfunction manifested by jaundice, hepatosplenomegaly, failure to thrive, anorexia, ascites, coagulopathy, and rickets. The disorder may actually begin in utero as evidenced by the presence of well-established cirrhosis with large regenerative nodules during infancy. The chronic form presents later in childhood with cirrhosis, renal tubular dysfunction, rickets, and hepatocellular carcinoma (HCC). Episodes of severe peripheral neuropathy occur in patients surviving infancy, leading to morbidity from severe pain and even mortality from respiratory failure.

Laboratory studies indicate significant compromise of hepatic synthetic function. Serum aminotransferase values are mildly to moderately elevated. Hypoglycemia is common, particularly in infants. Renal tubular dysfunction produces a Fanconi's syndrome with hyperphosphaturia, glucosuria, proteinuria, and aminoaciduria. Serum tyrosine and methionine concentrations are markedly elevated. Succinylacetone and succinylacetoacetate in the urine are typical and diagnostic of this disorder. Serum α-fetoprotein concentrations are often significantly elevated in affected infants and in cord blood, suggesting prenatal onset of liver disease. Histologic examination of the liver reveals fatty infiltration, iron deposition, varying degrees of hepatocyte necrosis, and pseudoacinar formation. Significant fibrosis may be present early in life with gradual evolution to multinodular cirrhosis, and the regenerative nodules mimic neoplasms in some patients (Fig. 47.8). HCC occurs frequently in older patients with cirrhosis. Livers of patients with HT often contain discrete nodules containing FAH activity. This mosaicism of enzymatic reactivity is due to somatic reversion to a normal genotype in these nodules. Molecular studies confirm correction of one of the disease-causing alleles in these nodules. These studies and other work in animal experiments suggest a strong selective advantage for FAH-expressing cells in an FAH-deficient liver. This information may be exploited in the future for gene therapy of HT.



▪ **Figure 47.8** Hereditary tyrosinemia. In this well-established cirrhosis, the regenerative nodules show variation in size and cytology. A nodule on the left has marked steatosis, whereas in the remainder of the specimen steatosis is absent.

The acute form of HT is usually fatal in the first year of life without therapy. Treatment with a diet restricted in phenylalanine, methionine, and tyrosine does not prevent progression of the liver disease or development of HCC. Liver transplantation reverses hepatic, neurologic, and most renal manifestations of the disease. Patients with cirrhotic nodules should be considered for transplantation because of the high risk of developing carcinoma. Treatment of HT has been revolutionized by the use of a metabolic inhibitor that acts early in the tyrosine degradation pathway (inhibiting 4-hydroxyphenylpyruvate dioxygenase) to prevent formation of the toxic intermediates succinyl acetoacetate (SAA) and succinyl acetone (SA). This inhibitor, (*2-[2-nitro-4-trifluoromethylbenzolyl]-1,3-cyclohexanedione*) *NTBC*, was first used in 1992 in five children with tyrosinemia. After 7 to 9 months, these patients had normal liver enzymes and coagulation studies, a decrease in α-fetoprotein levels, and no SA excretion. No toxicity was demonstrated. This report led to widespread clinical trials that largely reproduced the original experience, and more than 300 children with tyrosinemia have been treated, with subsequent stabilization of hepatic and renal function, improvement in growth and nutritional parameters, and delay or avoidance of liver transplantation. This drug has been licensed as nitisinone (trade name Orfadin). Although enthusiasm for this treatment has been somewhat tempered by the demonstration that NTBC administration corrects the hepatic and renal abnormalities in knockout mice deficient in FAH

activity but does not prevent all of the SA accumulation or the development of HCC, it is considered the standard of care for the treatment of HT, in addition to the dietary measures discussed in the preceding text (37). At present, the most prudent therapy for tyrosinemia is the institution of nitisinone and dietary therapy at the time of diagnosis. Close medical and radiographic monitoring is suggested for hepatic nodules that may herald early HCC, because a small percentage of children begun on therapy late in the course of disease have developed HCC despite treatment (38). Early liver transplantation is indicated in patients with severe neurologic crises.

Disorders of Bile Acid Metabolism

To date, several distinct inborn errors of bile acid biosynthesis have been recognized (39); these may be primary enzyme deficiencies or disorders that arise secondary to specific organelle dysfunction such as peroxisomal disorders. At present, two distinct major treatable disorders related to defective transformation of the steroid nucleus have been described: *Δ^4-3-oxosteroid 5β-reductase deficiency* and *3β-hydroxy-C_{27}-steroid dehydrogenase/isomerase deficiency*. The incidence of these defects is not known. In each, primary bile acid (cholic acid) synthesis is absent or markedly impaired, and urine, serum, and bile predominantly contain atypical bile acids. The cholestasis and liver injury are attributed to failure of synthesis of adequate amounts of the normal choleretic primary bile acids that are essential for the promotion and secretion of bile and/or the increased production of unusual primitive hepatotoxic bile acid metabolites. The unusual bile acids found in patients with defects in bile acid biosynthesis might cause cholestasis by inhibiting the canalicular ATP-dependent transport system for bile acids that constitute the rate-limiting step in the overall process of bile acid transport across hepatocytes.

The initial clue to the diagnosis is the finding of low serum concentrations of the primary bile acids in the presence of cholestasis. Impaired synthesis of bile salts may not be associated with either pruritus or elevated serum bile salt concentrations, because the normal end products are not synthesized. A high index of suspicion is required, because the presentation may be protean and diagnosis requires relatively specialized testing. Screening assays for these defects include gas chromatography and fast atom bombardment mass spectrometry of urine, serum, and/or bile. Confirmatory enzymatic and molecular assays exist for some of the defects.

Replacement bile acid therapy has been associated with reversal of hepatic injury and normalization of liver biochemical and histologic abnormalities (40). The rationale for bile acid therapy in inborn errors of bile acid biosynthesis is to replenish the bile acid pool and downregulate endogenous bile acid synthesis, thereby decreasing the production of toxic bile acid intermediates. Ursodeoxycholic acid (UDCA) presumably displaces toxic bile acids, improves bile flow, and offers cytoprotection.

Zellweger's (cerebrohepatorenal) syndrome is an example of a secondary defect in bile acid biosynthesis due to a primary defect in peroxisome development (41). Clinical manifestations include profound psychomotor retardation, hypotonia, a characteristic facies (narrow cranium, prominent forehead, hypertelorism, and epicanthic folds), cortical cysts of the kidney, and intrahepatic cholestasis. Hepatomegaly is usually present at birth and jaundice appears at 2 to 3 weeks of life. The hepatic histology includes hepatocellular damage with marked cholestasis, giant cell transformation, and pericellular fibrosis. Ultrastructurally, there is absence of peroxisomes (microbodies). Death occurs in most patients by 6 months of age. Diffuse micronodular cirrhosis is noted at autopsy.

A third type of genetic defect in bile acid metabolism is that of *bile acid transport defects*, which will be discussed subsequently in the chapter under the section on progressive familial intrahepatic cholestasis (PFIC).

Disorders of Bilirubin Metabolism and Excretion

Unconjugated hyperbilirubinemia is frequently encountered in infants. The causes are listed in Table 47.4. Normal hepatic clearance of bilirubin includes glucuronidation and carrier-mediated transport of bilirubin at the basolateral and canalicular membranes of the hepatocyte. Primary genetic abnormalities of these processes include Crigler-Najjar and Gilbert's syndromes that result from defects in glucuronidation (42) and Dubin-Johnson syndrome that results from a defect in canalicular excretion of conjugated bilirubin.

Gilbert's syndrome is the most benign of these disorders and probably represents a relatively common genetic phenotype. Between 2% and 10% of individuals have Gilbert's syndrome, which is the result of an alteration in the promoter for the bilirubin uridine diphosphophate glucuronyl transferase (*UDP-GT*) gene. The altered promoter is transcriptionally less active and leads to a relative deficiency of the enzyme. Clinically, this translates into a condition characterized by mild indirect hyperbilirubinemia (typically <5 mg/dL) without associated hemolysis or hepatocellular or canalicular injury. This may manifest as jaundice during times of stress and fasting. In addition, Gilbert's syndrome may explain many cases of prolonged "physiologic" jaundice in the newborn period (43). This entity has not been associated with any specific morbidity or

mortality. The liver biopsy reveals normal morphology. Gilbert's syndrome does not require treatment.

Table 47.4. Causes of Unconjugated Hyperbilirubinemia in Infants

Hemolytic diseases
Isoimmune (ABO or Rh incompatibility)
Congenital spherocytosis
Hereditary elliptocytosis
Red blood cell enzyme defects (e.g., glucose-6-phosphaste dehydrogenase)
Disorders of bilirubin conjugation or transport
Crigler-Najjar syndrome types 1 and 2
Gilbert's syndrome
Dubin-Johnson syndrome
Enclosed hematoma
Polycythemia
Diabetic mother

Twin–twin transfusion
Hypothyroidism
Sepsis
Intestinal obstruction
Breast milk jaundice

Crigler-Najjar syndrome has two forms, types I and II. Type I is the more severe, the result of a complete absence of bilirubin UDP-GT activity. This disease presents in the newborn period with severe indirect hyperbilirubinemia, necessitating continuous phototherapy and/or exchange transfusions. Analysis of bile reveals no bilirubin conjugates. Kernicterus is a serious potential complication for children with Crigler-Najjar syndrome type I and can occur at any age. Specific DNA testing can confirm the diagnosis of Crigler-Najjar type I, and can be utilized for prenatal testing. Phototherapy is the mainstay of therapy. Tin-mesoporphyrin, a synthetic heme analog with inhibitory activity against heme oxygenase, may decrease bilirubin production in affected individuals (44), but large-scale long-term studies have not yet been performed. Liver transplantation can be curative, although risk-benefit decisions for this approach can be complex (45). Preliminary reports of hepatocyte transplantation for this disorder have been disappointing. Gene therapy may ultimately be an attractive means of curing this disease.

Crigler-Najjar type II is the result of genetic abnormalities in the bilirubin *UDP-GT* gene that lead to partial activity. Hyperbilirubinemia of less than 10 mg/dL is usually observed and is typically responsive to cytochrome P-450–inducing compounds such as phenobarbital. Long-term treatment with phenobarbital is generally not recommended because it results in a cosmetic improvement but is associated with potential neurodevelopmental complications.

Dubin-Johnson syndrome is the genetic deficiency in the *cMOAT/MRP2* gene, which encodes the canalicular transporter of conjugated bilirubin (46). The disease is manifest by relatively mild conjugated hyperbilirubinemia (3 to 8 mg/dL) with no evidence of significant hepatocellular or canalicular injury. The liver, however, accumulates a dark green or black pigment within the pericanalicular cytoplasm of zone 3 hepatocytes. The pigment is coarsely granular and imparts a black discoloration to the gross liver. Analysis of urinary coproporphyrins reveals a preponderance of the isoform I. Like Gilbert's syndrome and Crigler-Najjar type II, this is a disease that is not associated with significant morbidity or mortality and as such does not require treatment.

Disorders of Fatty Acid Oxidation

Normal fatty acid metabolism in the liver is a highly complex process, which involves transport into hepatocytes and mitochondria and a series of distinct enzymatic steps that generate energy from fatty acid oxidation (FAO). During prolonged fasting, when glucose supplies are depleted, fatty acids are mobilized from adipose tissue, taken up by liver and muscle, and oxidized stepwise to acetyl-CoA, providing an important source of energy for cardiac and skeletal muscles. Abnormalities in fatty acid metabolism can lead to acute liver injury, as a result of both energy deprivation and the accumulation of highly toxic intermediary metabolites. At least 22 different clinical entities have been ascribed to distinct abnormalities in FAO (47). Clinical presentations include a "Reye's-like" syndrome with hypoglycemia, fatty liver, and coma; sudden, unexplained death; or skeletal- or cardiomyopathy with mild or severe liver disease. The typical case presents with elevated serum aminotransferase values and variable degrees of coagulopathy with normal bilirubin. Nonketotic hypoglycemia is a hallmark feature. Some form of stressor that includes fasting typically precedes the onset of symptoms. Both clinical manifestations and biochemical abnormalities may be intermittent. Isolated deficiency of mitochondrial long-chain fatty acid β-oxidation in the fetus may be associated with severe illnesses in heterozygote women during pregnancy. These include acute fatty liver of pregnancy (48); the hypertension/hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome; and hyperemesis gravidum.

Diagnostic testing is complex, and includes both nonspecific screening assays as well as more specific enzymatic and molecular tests. Initial evaluation includes assays of plasma carnitine, acylcarnitines, free fatty acids, and urine organic acids and acylglycines. An important diagnostic tool is tandem mass spectrometry, which can identify diagnostic abnormal acylcarnitines in most disorders using random plasma samples or filter paper blood spot cards. This technique has been adapted for expanded newborn screening. Most diagnostic assays must be performed during illness, as many

of the metabolites will clear with treatment. These preliminary assays are followed by specific enzymatic assays in fibroblasts, with DNA diagnostic confirmatory tests, when possible (49).

Long-term treatment is directed at halting fat catabolism. This can be achieved by intravenous administration of 12 to 15 mg/kg per minute of glucose. The principle of long-term treatment is to avoid reliance on FAO by preventing prolonged fasting and using low-fat diets supplemented, if necessary, by carbohydrate snacks. The benefits of carnitine administration and specific dietary fat restrictions or supplementation are controversial. If a diagnosis cannot be made before the development of irreversible hepatic injury, liver transplantation may be considered, but care must be taken to ensure that there is no evidence of systemic disease that would not be addressed by hepatic replacement.

Primary Mitochondrial Hepatopathies

The liver is a major target organ in inherited defects of mitochondrial oxidative phosphorylation. Neonatal and early childhood hepatic failure have been associated with defective activity of respiratory chain complexes and oxidative phosphorylation (50), but liver disease of varying severity and at different ages has also been reported (51). Neonatal liver failure occurs in association with deficiency of complex IV (cytochrome *c* oxidase) and of complexes I and III. Evidence of synthetic failure is prominent with hypoglycemia, hypoproteinemia, hyperbilirubinemia, hyperammonemia, and coagulopathy. Lethargy, hypotonia, vomiting, and poor feeding are also seen. Prenatal onset is suggested in some patients by the occasional occurrence of fetal hydrops and congenital ascites. An important diagnostic feature in these patients is lactic

acidosis and an elevated molar ratio of plasma lactate to pyruvate (normal <20:1). Histologic features of the liver include microvesicular and macrovesicular steatosis, often with increased mitochondrial density giving an oncocytic appearance to the hepatocytes. Cholestasis, bile ductular proliferation, fibrosis, or even cirrhosis may be present. Activities of mitochondrial respiratory chain enzymes can be measured in affected tissues.

Because heteroplasmy for mitochondrial DNA (mtDNA) mutations is not uniform in all tissues, patients may present exclusively with liver disease or with variable involvement of other organ systems (52). Most commonly, this includes varying degrees of neuromuscular involvement, including hypotonia, cardiomyopathy, ophthalmoplegias, diabetes mellitus, deafness, nephropathy, intestinal pseudo-obstruction, and pancreatitis. This issue is important, because patients may undergo successful liver transplantation only to have later onset of severe neuromuscular or cardiac disease (53). The spectrum of respiratory chain disorders is sufficiently broad that some patients are now being recognized with a more chronic course or onset later in infancy or childhood. Alpers' disease is one such disorder; it is characterized by progressive neuronal degeneration and cirrhosis of the liver in childhood.

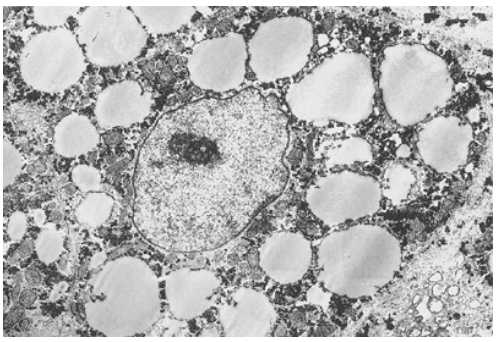
Point mutations, deletions, and rearrangements in mitochondrial or nuclear DNA have been detected in patients with neonatal liver failure caused by respiratory chain defects (52). Some children with severe liver disease have been found to have the mtDNA depletion syndrome in which there is a generalized reduction of otherwise normal mtDNA molecules in affected organs. Autosomal recessive, maternal, and possibly X-linked modes of inheritance have been observed or proposed. The prognosis for patients with acute liver failure secondary to mitochondrial disorders is extremely poor. There is no proven medical therapy, although supplementation with mitochondrial cofactors such as coenzyme Q10 and antioxidants theoretically, may be beneficial. Liver transplantation has been successful in patients whose disease is restricted to the liver.

Mitochondrial injury—Reye's syndrome

Reye's syndrome (RS) is a systemic illness characterized by acute liver disease and hyperammonemic encephalopathy (54). RS had a peak incidence in the 1960s and 1970s; only rare cases have been reported since 1985 (55). Although the cause of this disease was never fully elucidated, the stereotypic presentation after routine childhood viral illnesses, such as varicella and influenza, and the association with aspirin ingestion, raised the possibilities of immune-mediated and/or toxic pathogenesis. The reasons for the decreasing incidence of RS are not clear, but may in part be due to the avoidance of aspirin administration to children with viral illnesses.

This disorder shares many features with mitochondrial hepatopathy, and indeed, abnormal mitochondria are an important ultrastructural feature. The microvesicular steatosis characteristic of this disorder indicates a mitochondrial energy-generation defect. Children with RS develop vomiting within a few days of the antecedent illness; the vomiting is not due to gastrointestinal upset, but more likely to the earliest stages of encephalopathy. In the mildest cases, the vomiting is associated with minimal neurologic changes such as lethargy, and resolves spontaneously or with intravenous glucose. In the more severe cases, vomiting is followed quickly by progressive obtundation and the stages of metabolic coma. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values are typically moderately to markedly elevated. Prolonged

prothrombin time is common, but in contrast to fulminant hepatitis jaundice is not noted. Hyperammonemia is usually found at presentation; the average value is approximately 350 g/dL, but the level does not correlate with the severity of the encephalopathy.



▪ **Figure 47.9** Microvesicular steatosis. Ultrastructutal appearance of hepatocyte with microvesicular steatosis. The nucleus remains at the center and fat vesicles are numerous, small and uniform.

The liver is enlarged but not firm; it is characterized histologically by extensive accumulation of microvesicular fat, but inflammation, necrosis, and cholestasis are absent. EM reveals marked microvesicular steatosis (Fig. 47.9) and characteristic mitochondrial changes with swelling of matrix, dissolution of cristae and intramatrical granules, and ameboid shapes. The hepatopathy resolves over the first several days of the illness, and the prognosis is determined by the central nervous system component of the illness. Treatment is directed at maintaining metabolic homeostasis with glucose administration, and minimizing intracranial hypertension with maneuvers such as hyperventilation and mannitol infusion.

There are pediatric disorders that may mimic RS, with liver disease and encephalopathy. Children who present sporadically with vomiting, lethargy with progressive coma, and biochemical evidence of hepatocellular injury may have systemic viral infections with multisystem involvement, toxin exposure, or inborn errors of metabolism. The genetic/metabolic disorders that are most likely to be confused with RS are FAO defects and urea cycle defects. These should be suspected in very young children, or in those who have had recurrent episodes of hepatopathy and encephalopathy, especially if preceded by periods of fasting or other metabolic stress.

Mitochondrial injury—copper overload

The mitochondrion is a major intracellular target for copper toxicity. Accumulation of copper in the hepatic mitochondria leads to oxidative stress (increased free radical generation) with subsequent lipid peroxidation and oxidative alterations of

thiol-containing proteins. Oxidant damage in hepatic mitochondria also leads to deletions in mtDNA in young adults with Wilson disease (52). Therefore, dysfunction of hepatic mitochondrial electron transport may be an important factor in the pathogenesis of liver dysfunction and liver failure in copper overload states. Treatment to reduce oxidative stress (e.g., antioxidants) may protect the mitochondria from injury.

Wilson disease, the most common genetic disorder associated with copper overload and mitochondrial injury, is discussed in Chapter 35. Wilson disease should be considered in any child with an unexplained hepatic, neurologic, or psychiatric illness. The clinical presentation is highly variable. Although patients as young as 2 years have presented with liver disease, symptoms are rarely evident before 5 years of age. Younger children, identified by family screening or after evaluation of abnormal liver biochemical tests, are often asymptomatic. Patients younger than 20 years tend to present predominantly with hepatic manifestations. Kaiser-Fleischer rings are often absent in younger patients presenting only with liver disease.

Several non-Wilsonian copper overload syndromes have been described in children. The best described is *Indian childhood cirrhosis* (ICC). A unique familial form of liver disease, ICC, confined primarily to the Indian subcontinent, affects Hindu children (primarily boys) below 3 years (56). It is characterized by a striking accumulation of copper-associated granules within hepatocytes. Affected children present insidiously with hepatomegaly, abnormal stools, and behavioral changes, such as increased appetite, excessive irritability, and disturbed sleep patterns. Jaundice is uncommon; ceruloplasmin levels are normal. ICC is progressive and results in hepatocellular failure and death. Investigators who believe that ICC is a primary dietary copper toxicity cite the widespread use of brass cooking utensils among middle-class Indian families and the increased rate of absorption and storage of copper in infants compared with adults. Those who favor an inborn error of metabolism as the cause of ICC cite the 25% to 30% recurrence rate within families and other epidemiologic studies reporting a strong genetic component. Children older than 3 years rarely, if ever, develop typical ICC in spite of a similar environment and diet. Therefore, immaturity of hepatocytes during that critical phase of high dietary intake makes them prone either to accumulate copper excessively or fail to excrete it through the bile. In composite, ICC may represent a genetic disorder that requires an environmental influence to be manifest. The liver biopsy reveals hepatitis reminiscent of alcoholic liver disease with marked ballooning of hepatocytes, Mallory hyaline, and neutrophilic infiltrate. Copper-binding

protein accumulates in hepatocytes. The process progresses to venosclerosis and pericellular fibrosis, leading to cirrhosis. Preliminary therapeutic studies of D-penicillamine have led to clinical, functional, and histologic improvement (57). In contrast to Wilson disease, therapy can apparently be discontinued after 2 years without reaccumulation of copper in the liver.

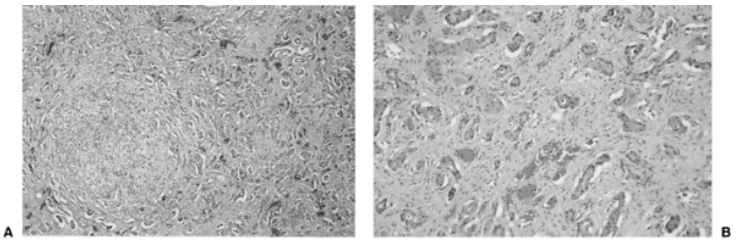
α_1-Antitrypsin Deficiency

α_1-Antitrypsin deficiency is an important genetic liver disease in children. In some pediatric centers, it is the most common genetic disease requiring liver transplantation. This disorder is discussed in detail in Chapter 37.

Cholestatic jaundice occurs in approximately 10% to 15% of infants with the protease inhibitor ZZ (PIZZ) phenotype. Patients rarely present with signs of advanced liver disease. Another 40% to 50% of homozygous infants show asymptomatic abnormal liver biochemical test results in the first months of life. Giant cell hepatitis is a typical histologic finding in the neonate. Bile ductular proliferation may be observed initially; occasionally, paucity of bile ducts is found later. The periodic acid-Schiff positive and diastase-resistant α_1-antitrypsin inclusions within periportal hepatocytes are hallmarks of the disorder but are not prominent before 4 months of age. The outcome of neonatal liver disease related to α_1-antitrypsin deficiency is variable. In most infants, jaundice clears by 4 months of age. Patients may present with cirrhosis later in childhood and are at risk for HCC. There is no specific treatment for α_1-antitrypsin deficiency. Liver transplantation is curative for patients progressing to end-stage liver disease; the recipient assumes the Pi type of the donor organ.

Neonatal Hemochromatosis

Neonatal hemochromatosis (NH) has also been called *neonatal iron storage disease* (58). This disorder, characterized by marked hepatic and extrahepatic hemosiderosis, is a form of neonatal liver failure that is characterized by an early in utero onset. Whether this disease represents a single pathophysiologic entity or is a common pathologic end-point of a number of diseases that lead to in utero liver failure is not known. NH is unrelated to hereditary hemochromatosis or the *HFE* gene that is often defective in that disease. NH does not appear to be the result of a primary abnormality in fetal iron metabolism. Infants with NH have very high mortality unless prompt treatment and/or liver transplantation is undertaken.



▪ **Figure 47.10** Neonatal hemochromatosis. **A:** Marked and diffuse interstitial fibrosis with pseudoacinar transformation of hepatocytes. A regenerative nodule is present at the lower left. **B:** At higher magnification the interstitial fibrosis (interstitial cirrhosis) is better seen.

The initial clinical presentation of NH can be subtle. The findings of cholestatic jaundice with coagulopathy and/or ascites at birth should prompt diagnostic evaluations. Supporting biochemical features include thrombocytopenia, hypoalbuminemia, hypoglycemia, hyperammonemia, and high iron saturation and serum ferritin levels. Diagnosis is dependent on documentation of hepatic insufficiency and extrahepatic siderosis with no other apparent etiology of the liver failure.

Extrahepatic siderosis can be demonstrated by either biopsy of a minor salivary gland or magnetic resonance imaging of the pancreas and/or heart. Analysis of both these studies requires assessment by a specialist experienced in the special applications of these diagnostic tests. Liver histology, when available, reveals nonspecific findings with marked diffuse hepatocellular damage, significant hepatocellular loss with parenchymal collapse, and well-established pericellular fibrosis or cirrhosis (Fig. 47.10A,B).

Immediate referral of infants with NH to a center experienced in liver transplantation in infants is advised. Medical therapy consists of a combination of antioxidants (vitamin E in the form of tocopheryl polyethylene glycol succinate, selenium, and *N*-acetyl cysteine), membrane stabilizers (prostaglandin $E_{1\alpha}$), and an iron chelator (deferoxamine). The efficacy of medical therapy alone has been questioned, but it

appears at a minimum to stabilize infants in preparation for liver transplantation, which has been successful in very small infants with NH (59). Recurrence of the disease post-transplant has not been reported.

The inheritance of NH is complex and in many circumstances is associated with a recurrence rate in siblings that exceeds that expected for either an autosomal recessive or dominant pattern. For these reasons, ascertainment of a diagnosis of NH is of critical importance for family counseling and for monitoring subsequent pregnancies. Recently, an alloimmune etiology for this disorder has been hypothesized, and, on the basis of the hypothesis, high-dose immunoglobulin has been provided during subsequent pregnancies to 15 women who had previously delivered affected infants. A highly significant difference in outcome and survival was demonstrated in the newborns compared with those from prior pregnancies (60).

Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis PFIC is a heterogeneous group of disorders with some common features: Jaundice due to intrahepatic cholestasis beginning anywhere from infancy to late childhood, severe pruritus, growth failure, and a genetic pattern most consistent with autosomal recessive inheritance. Patients with PFIC present with pruritus and jaundice early in life. A subset of infants has a phenotype indistinguishable from that of NH (discussed in preceding text) (61). Initially the jaundice may be episodic, but the biochemical cholestasis persists and eventually the patient becomes permanently icteric. In some types of PFIC, the biochemical profile differs from that of other forms of cholestasis in that serum γ-glutamyl transpeptidase (GGT), which may be two to three times the normal initially, becomes normal. Serum cholesterol levels are usually normal as well. The hepatic histology early in the course may show hepatocanalicular cholestasis, giant cell transformation, and ballooning of hepatocytes around terminal hepatic venules. Paucity of interlobular bile ducts and bile duct epithelial degeneration are prominent findings. With progression, fibrosis is initially centrilobular and later causes central to portal bridging. A characteristic pattern consists of diffuse pericellular fibrosis, severe cholestasis, and pseudoacinar transformation of hepatocytes (62).

Complications include fat-soluble vitamin deficiencies, growth retardation, delay in sexual maturation, infections related to cholestasis, cholelithiasis, and chronic pancreatitis. HCC may develop in children with advanced cirrhosis, even before 3 years of age.

Three distinct types of PFIC have been described so far. Type 2 is mentioned in the preceding text under the section on bile acid metabolism disorders, because it has been found to be due to an inborn error in canalicular bile salt transport.

Low serum γ-glutamyl transpeptidase and cholesterol levels help to differentiate PFIC types 1 and 2 from other chronic cholestatic disorders in children. PFIC type 1, previously known as *Byler's disease*, is a syndrome in which chronic, unremitting cholestasis develops early in life. The gene *ATP8B1* encodes a protein called *FIC1*. It was mapped to the same locus as that for benign recurrent intrahepatic cholestasis (BRIC) at chromosome 18q21-q22, and has been cloned (63). *ATP8B1* encodes for a P-type ATPase that may be involved in ATP-dependent aminophospholipid transport. Although the genetics of FIC1 disease are complex, various mutations have been detected in patients affected with PFIC-1, BRIC, and a unique geographic form, Greenland familial cholestasis (64). *ATP8B1* is expressed in several epithelial tissues, particularly intestine and bile duct cells, but not in hepatocytes. Manifestations of the deficiency in these other tissues are being elucidated. Progression to cirrhosis and liver failure usually occurs by 3 to 4 years of age but may develop in the neonatal period.

Molecular transporters of bile acids are found on both the canalicular and basolateral hepatocyte membranes. Canalicular excretion of bile salts is mediated by the bile salt excretory protein, bile salt export pump (BSEP), encoded by the gene *ABCB11* or ATP-binding cassette B11. A primary genetic defect in BSEP, which maps to 2q24, leads to a disorder called *progressive familial cholestasis type 2* (*PFIC-2*). This defect results in progressive cholestasis characterized by severe pruritus, markedly elevated serum bile acid concentration, normal serum cholesterol concentration, and normal GGT values (42). Biliary bile acid concentrations are extremely low. Liver histology early in the course of the disease may reveal giant cell transformation and ductular proliferation. Cirrhosis and end-stage liver disease may evolve relatively quickly. Differentiation of this disease from PFIC-1 may be difficult, but it is critically important because primary defects in canalicular transport of bile acids may be cured by liver transplantation, although PFIC-1 may not. Defects in the basolateral transport of bile salts have not been definitively identified, although this is suspected to be the cause of a relatively rare form of cholestatic liver disease characterized by isolated elevations of serum bile salts and severe pruritus.

There is a third variant of PFIC, *PFIC-3*, in which patients have high serum GGT values and low concentrations of phospholipids in bile. These children develop severe liver disease characterized by diffuse hepatocellular damage, cholestasis, inflammation of portal tracts, bile ductular proliferation, and fibrosis. Immunohistochemical techniques have revealed the absence of canalicular staining for a gene called *MDR3*

or *ABCB4*, which encodes a phosphatidylcholine flippase, in liver tissue from affected patients. Genomic DNA analysis has demonstrated several mutations in the *MDR3* gene, in this disorder (65). In addition to the severe, progressive cholestatic disorder of childhood caused by mutations in this gene, a link between PFIC-3 and intrahepatic cholestasis of pregnancy has been established. Heterozygous women carrying fetuses affected with distinct nonsense or missense mutations in *MDR3* were found to have typical recurrent episodes of cholestasis during pregnancy (66). A similar link was recently reported in adults with symptomatic gallstone disease (67), demonstrating the role of this transporter in the maintenance of normal ratio of cholesterol to phospholipid in bile.

PFIC is generally refractory to medical therapy, although UDCA has proven of benefit in some forms. Prompt surgical management with partial diversion of the bile flow may arrest progression of hepatic fibrosis and improve pruritus, growth and school attendance (68). Liver transplantation is reserved for patients who have cirrhosis.

Miscellaneous Familial Cholestatic Syndromes

A number of familial, presumably genetic, syndromes of childhood intrahepatic cholestasis have been described in ethnic populations. Whether they are variants of one disorder, expressed in geographically different kindreds, or clinically similar but genetically distinct diseases, has not been elucidated in most instances.

"Norwegian cholestasis" has been reported in patients of Norwegian extraction and is characterized by lymphedema of the lower extremities and recurrent bouts of intrahepatic cholestasis that begin within the first 3 months of life (69). North American Indian cholestasis is restricted to a tribe of native North Americans from northwest Quebec (70). The disease often presents with neonatal cholestasis, with the striking feature of telangiectasias of the cheeks ("papermoney skin"). Greenland Eskimo cholestasis, also limited to a well-defined ethnic group, presents at birth or within the first 3 months of life with permanent jaundice and pruritus (71). A unique missense mutation in the *ATP8B1* gene has been implicated (64), linking this disorder to PFIC-1 and BRIC.

The types and features of the various forms of PFIC and other genetic cholestasis disorders are listed in Table 47.5.

Viral Hepatitis

Viral hepatitis is extensively discussed in Part V, Chapters 26, 27, 28, 29, 30 and 31. Although the prevalence of viral hepatitis is much lower in children than in adults, it is important to identify infected children to interrupt horizontal and community transmission, detect liver disease, intervene to minimize progression, and design a strategy for immunization when available. This requires understanding of risk factors important in children. The natural history of some kinds of viral hepatitis in children may be more benign than infection acquired in adulthood, but childhood cases contribute disproportionately to the overall disease burden in some instances. Identification of appropriate pediatric candidates for treatment and definition of optimal therapy in these children represent a unique challenge. Because viral hepatitis is covered in other sections of the book, only selected aspects of special pertinence to the pediatric population will be discussed here.

Hepatitis A

Outbreaks of hepatitis A virus (HAV) have been traced to day care centers, where unsanitary practices involving infants and children have been implicated in viral transmission. The severity of disease caused by HAV infection is inversely correlated to age; icteric HAV infection occurs in approximately 10% of children younger than 5 years, but in up to 80% of adults. Severe liver dysfunction such as coagulopathy, marked cholestasis, and encephalopathy, is rare in childhood, but more common in adolescents and adults.

Hepatitis A vaccines are licensed for children 2 years of age and older. Routine HAV vaccination is currently recommended for children in the United States who live in "endemic" areas (rates of HAV infection twice the national average, i.e., >20 cases per 100,000 population), and should be considered for those living in areas of "intermediate" incidence (rates of HAV infection 10 to 20 cases per 100,000 population) (72). This has proven to be a cost-effective strategy (73). Otherwise, the indications are the same as for adults: Anticipated travel to an HAV-endemic area, underlying chronic liver disease, or in the control of an outbreak. In children younger than 2 years in whom vaccine efficacy and safety have not been adequately determined, immunoglobulin is recommended if needed to prevent infection. Intramuscular immunoglobulin is efficacious and safe, but may interfere with the immunologic response to several live-virus vaccines including mumps, measles, rubella (administered individually or in combination) and varicella. Accordingly, mumps, measles, and rubella immunization should be delayed by 3 months and varicella vaccination by 5 months after the administration of immunoglobulin.

Postexposure prophylaxis is recommended for susceptible personal contacts of an HAV-infected child, such as a household member (72). In addition, day care staff and attendees in close contact with an HAV index

case should receive passive immunization. Administration of immunoglobulin is not routinely indicated for exposures arising in the school setting.

Table 47.5. Genetic Disorders Associated with Intrahepatic Cholestasis During Infancy and Childhood

Genetic disorder	Associated findings	GGT	Pruritus	Serum bile acid concentration	Inheritance	Chromosomal location	Gene defect
Alagille syndrome	Cardiac, vertebral, facial, growth abnormalities	High	Yes	High	Autosomal dominant	20p12	*JAG1* (encodes Jagged 1)
Bile acid synthesis defects[a]	None		No	Low	Autosomal recessive	Unknown	(see subsequent text)
PFIC-1	Diarrhea	Low	Yes	High	Autosomal recessive	18q21-q22	*ATP8B1* (encodes FIC1)
PFIC-2	Growth failure	Low	Yes	High	Autosomal recessive	2q24	*ABCB11* (encodes BSEP)

PFIC-3	Later presentation	High	Yes	High	Autosomal recessive	7q21.1	*ABCB4 (MDR3)*
Norwegian cholestasis	Lymphedema	High	NR	NR	Autosomal recessive	Unknown	Unknown
North American childhood cholestasis	Telangiectasia of the cheeks	High	NR	NR	Autosomal recessive	Unknown	Unknown
Greenland cholestasis	Thrombocytosis, subcutaneous bleeding	NR	Yes	NR	Autosomal recessive	18q21-q22	*ATP8B1* (specific mutation)

[a]Bile acid synthesis defects include 3β-hydroxy-C27-steroid dehydrogenase/isomerase deficiency, Δ^4-3-oxosteroid 5-β-reductase deficiency, oxysterol 7α-hydroxylase deficiency, and others.
GGT, γ-glutamyl transpeptidase; PFIC, progressive familial intrahepatic cholestasis; NR, not reported; BSEP, bile salt export pump; MDR3, multidrug resistance gene.

Hepatitis B

Children at risk for hepatitis B virus (HBV) infection are neonates born to infected mothers, children who live in subpopulations with high endemicity (such as groups of immigrants from HBV-endemic areas), children adopted from these areas, and adolescent intravenous drug users. In the United States, children and adolescents comprise approximately 15% to 20% of new HBV infections annually. The clinical course and natural history of HBV infection is variable and depends on the age at which infection occurs. HBV infection during the perinatal period, infancy, and early childhood is rarely symptomatic, whereas adolescents and adults commonly develop symptoms. HBV acquisition during infancy results in the development of a chronic carrier-state in more than 90% of cases, characterized by persistence in serum of hepatitis B surface antigen (HBsAg), high-level HBV DNA and minimal biochemical or clinical evidence of liver disease. In contrast, acute HBV infection gradually resolves without chronic sequelae in more than 90% of infected adults. Although the precise mechanisms that underlie these age-related differences in the natural history of HBV infection are unknown, experimental evidence suggests that immune tolerance to viral antigens may play an important role.

Children who are candidates for treatment are those with serologic evidence of HBV infection for at least 6 months. The criteria include detectable serum HBsAg, HBeAg, and/or HBV DNA (in case of HBeAg negative HBV infection), and consistently abnormal ALT values. A liver biopsy should be obtained before the start of therapy to provide evidence of chronic hepatitis, to stage the disease, and to rule out other processes. In most studies performed before the high prevalence of HBeAg negative infections was appreciated, seroconversion from HBeAg to anti-HBe was used as the primary outcome variable for response to therapy. Therapeutic trial results must be compared to the "background rates" of spontaneous seroconversion. Other treatment responses that are frequently reported include decrease in serum HBV DNA to levels undetectable by non-polymerase chain reaction (PCR) assays, and normalization of ALT values.

There are two licensed medications for treatment of chronic HBV in adults and children in the United States:Interferon α (IFN-α) and lamivudine.

IFN-α induces sustained viral remission (disappearance of serum HBV DNA and HBeAg) in 25% to 40% of children with chronic hepatitis B (74). IFN-α is generally well tolerated by children; the most common adverse event described is a flu-like syndrome characterized by fever, headaches, chills, and myalgias, which generally resolve within the initial 2 to 3 doses. Other reported side effects include bone marrow suppression, neuropsychiatric disorders (depression and anxiety), fatigue, diarrhea, anorexia, and weight loss (75).

A randomized, double blind, placebo-controlled multicenter trial of lamivudine for 52 weeks in children with chronic HBV has been done (76). Twenty-three percent of children who received lamivudine cleared HBV DNA and lost HBeAg from serum compared with 13% of the placebo group ($P < 0.05$). Of those children who responded to the first year of treatment, 82% had sustained response at 6 months, although they had received no further treatment. As with IFN, children with higher ALT values and higher histology activity index scores at baseline had greater likelihood of virologic response (77). Prior receipt of IFN did not affect the response rate. Lamivudine did not interfere with growth, and side effects were the same in the lamivudine and placebo groups. Lamivudine treatment in adults has been complicated by the development of viral resistance, and the same has been true in children. The HBV in 19% of children had developed resistance to lamivudine after 1 year. As lamivudine is so well tolerated, many patients, including those with advanced liver disease in whom IFN is contraindicated, may be considered for treatment. It has been suggested that the medication should be continued until HBeAg disappears from the serum, anti-HBe appears, or, if neither occurs, HBV DNA becomes persistently undetectable. This strategy has not been validated in children. Although as an oral medication with a satisfactory safety profile lamivudine is an attractive medication for children, the optimal duration of therapy has not yet been defined. Newer agents such as pegylated IFN and other nucleoside analogs have not yet been studied in children with chronic HBV infection.

Although infants and children who are long-term HBV carriers have little clinical and histologic evidence of liver disease, they are at high risk to develop cirrhosis and HCC. Because children have minimal liver disease, they often do not benefit from currently available antiviral therapy. Although screening strategies to detect early HCC are evolving, periodic determinations of serum α-fetoprotein and serial abdominal sonography, as recommended for adults, seem reasonable approaches for children with chronic HBV infection.

HBV infection can be effectively prevented after exposure by the administration of hepatitis B immunoglobulin (HBIG). This preparation is indicated in the prevention of perinatal transmission from HBV-infected women, and should be given to their

neonates in the first hours of life. Completion of immunoprophylaxis in this setting is accomplished with active immunization with one of the two licensed HBV vaccines. Following

the initial failure of immunization strategies, which targeted high-risk individuals, vaccination is now recommended for all newborns, all preadolescent children, and adults at high risk (78,79). The vaccine is safe and effective in children, including premature infants (80,81). Concerns about mercury exposure in neonates and young children caused by thimerosal (used as vaccine preservative) prompted the development of preservative-free formulations.

Hepatitis C

In the United States, the estimated prevalence of hepatitis C virus (HCV) infection in children aged 18 years or younger is 0.1% to 0.2%, as compared with 1.8% in adults (82). Because HCV is a parenterally transmitted pathogen, children at risk include neonates born to HCV-infected mothers and recipients of transfusions of blood and blood products before 1992. The routine screening of blood for HCV has resulted in a dramatic decline in transfusion-associated infection, and perinatal exposure has become the major mode of transmission to children. The general incidence of transmission from HCV-viremic mothers is approximately 5% (83,84,85); active untreated human immunodeficiency virus (HIV) coinfection raises this risk substantially (86,87). Risk factors that may increase the likelihood of perinatal transmission include prolonged rupture of amniotic membranes and the use of internal fetal scalp monitors (83). There is as yet no evidence to indicate breast feeding as an additional risk. Current recommendations regarding perinatal HCV transmission are indicated in Table 47.6. HCV antibodies present in newborns are typically derived from the maternal serum, so testing after 15 months of age is indicated for the detection of HCV infection in these children.

Table 47.6. Guidelines Regarding Perinatal Hepatitis C Virus Infection

RECOMMENDED (supported by data)
Targeted testing of pregnant women who have risk factors for HCV
Aggressive treatment of HIV in coinfected pregnant women
Testing of infants for anti-HCV when they are 12 to 15 months of age
SHOULD BE CONSIDERED (suggested by data)
Avoid internal fetal scalp monitoring during labor
Deliver infant within 6 hours of rupture of membranes
NOT RECOMMENDED (no data to support)
Universal testing of pregnant women
Elective cesarean delivery
Avoidance of breast-feeding
Immunoglobulin administration to newborns

HCV, hepatitis C Virus; HIV, human immunodeficiency virus.

The natural history and pathobiology of HCV infection in infants and children is incompletely understood, but HCV infection leads to chronic liver disease in some children. It is generally mild, although aggressive liver disease with fibrosis and even cirrhosis can occur. Extrahepatic manifestations such as cryoglobulinemia, membranoproliferative glomerulonephritis, and vasculitis commonly seen in adults are seldom reported in children. Histologic findings of chronic hepatitis C in children are similar to those in adults (88,89).

If, when, and how to treat children with chronic hepatitis C is at present uncertain and controversial (90). Spontaneous viral remission may occur in the first couple of years after infection, but only in a minority of cases. Liver disease may progress silently in some children. Therefore, it is reasonable to consider treatment for children infected with HCV. The combination of IFN and ribavirin has been licensed for children aged 3 years and above. The use of multiple treatment regimens in mostly small and uncontrolled clinical trials of IFN-α in children with chronic hepatitis C makes direct comparison to adult data difficult. However, it is clear that children may have sustained virologic response to treatment at rates that are at least comparable to those in adults. The safety and efficacy of the combination of pegylated IFN and ribavirin are currently being evaluated in a large pediatric trial.

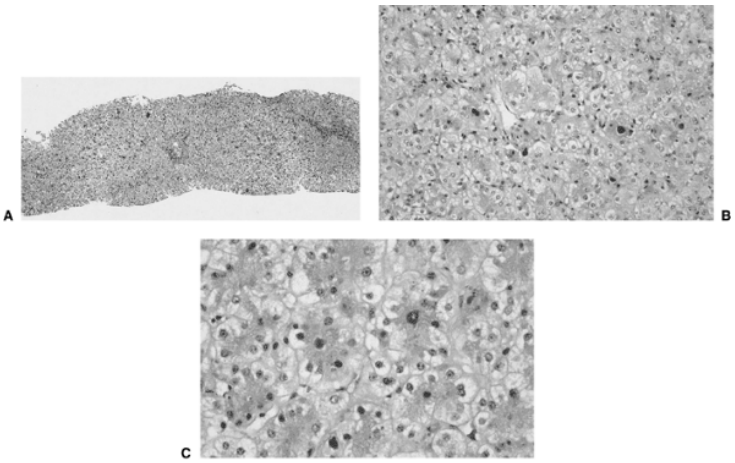
Epstein-Barr Virus

Epstein-Barr Virus (EBV) is a DNA virus that is transmitted primarily by close person-to-person contact with infected secretions, particularly saliva. After initial replication in epithelial cells of the oropharynx, EBV disseminates throughout the reticuloendothelial system by propagating in B lymphocytes. Liver involvement is common in childhood EBV infection; it may be manifested by hepatosplenomegaly, liver tenderness, modestly elevated aminotransferase values, and, occasionally, jaundice. The course of liver disease is usually mild and self-limited but occasionally can be severe and protracted, particularly in immunocompromised hosts. Fulminant hepatitis due to EBV infection is rare. Although liver biopsy is generally not indicated in EBV infection, it should be considered in severe cases with atypical features. Characteristic histologic findings that support the diagnosis of EBV include varying degrees of portal and lobular inflammation and sinusoidal infiltration by mononuclear cells (91). Management of EBV hepatitis is usually supportive although short courses of prednisone may be indicated in select children with severe or prolonged illness. Antiviral agents have no proven benefit in the treatment of EBV infection in immunocompetent patients.

Other Viruses Associated with Hepatitis

Other human herpesviruses, including cytomegalovirus (CMV) and human herpesvirus (HHV) types 1, 2, and 6, may be associated with liver disease, predominantly in neonates and infants (92). The clinical course of these infections can be severe in neonates and immunocompromised children. Parvovirus B19 is a small single-strand DNA virus that causes erythema infectiosum and has been associated with hydrops fetalis and aplastic anemia. Although liver involvement is seldom seen in these conditions, parvovirus B19 infection has been implicated in multisystem disease (93) and fulminant liver failure in children (94), and acute hepatitis in adults. Infections with paramyxoviruses, such as mumps and measles, very occasionally involve the liver and usually in the context of generalized disease. Coxsackie virus and echoviruses are

enteroviruses, they occasionally cause neonatal liver failure (95).



▪ **Figure 47.11** Total parenteral nutrition effect. **A:** At low power, this needle biopsy reveals centrilobular ballooning of hepatocytes. **B:** Centrilobular hepatocytes show pseudoacinar transformation and cholestasis. A terminal hepatic vein is seen at the center. **C:** At higher magnification the pseudoacinar transformation and canalicular cholestasis are better seen.

Liver Disease Associated with Parenteral Nutrition

There are three distinct but overlapping hepatobiliary syndromes associated with TPN discussed comprehensively in Chapter 13. Gallbladder sludge and gallstones may occur at any age but are rarely symptomatic. Older children and adults may develop hepatic steatosis or steatohepatitis. In infants, the typical complication is cholestasis or cholestatic hepatitis (96). It is characterized histologically by predominantly centrilobular hepatocellular damage with ballooning, cholestasis, varying degrees of steatosis, portal mixed inflammatory infiltrate with bile duct proliferation, and portal and pericellular fibrosis (Fig. 47.11A–C). The incidence of cholestasis associated with parenteral nutrition varies from 7% to 50%. The frequency increases with younger gestational age, lower birth weight, and longer duration of parenteral nutrition. Most cases occur from 2 to 10 weeks after starting TPN, and 90% of infants develop cholestasis after 13 weeks (97). TPN-associated liver injury in infants is characterized by hepatomegaly and jaundice. The most widely accepted laboratory indicator is a rising conjugated bilirubin level in a patient who has received TPN for at least 2 weeks. The ALT peaks between the second and fourth week of continuous TPN and then declines, whereas the bilirubin and alkaline phosphatase (AP) continue to increase and remain elevated for the duration of TPN. Patients who cannot be fed enterally and require continued TPN despite cholestasis may develop cirrhosis. Extreme short bowel syndrome may shorten

this period to as early as 6 months. Infants with necrotizing enterocolitis have a sevenfold increased risk for cholestasis. In fact, end-stage liver disease has replaced catheter-related sepsis and malnutrition as the leading cause of death in TPN-dependent infants with short bowel syndrome. HCC has been reported even in noncirrhotic patients (98,99).

A single etiology for TPN-associated cholestasis has not been identified. Factors that have been associated with TPN-related liver disease include immaturity of the enterohepatic circulation, perinatal insults, toxins, nutrient deficiency, contaminants, substrate imbalance, absence of enteral intake, and infection.

When biochemical evidence of cholestasis or hepatocyte injury is found, other causes of liver disease should be excluded. Avoidance of TPN, when possible, is the best approach. In cases where its use is mandatory, it is important to utilize enteral alimentation as soon as possible, even if only for partial or minimal feeding. Dosages of trace elements such as copper and manganese that are excreted through the biliary tree should be adjusted in TPN solutions for patients with cholestatic liver disease. Discontinuation of TPN before development of frank cirrhosis results in substantial improvement in the histologic changes in the liver.

Autoimmune Hepatobiliary Disease

Autoimmune Hepatitis

Although autoimmune hepatitis (AIH) is the subject of Chapter 31, there are some special features and types of AIH that are of special relevance to the pediatric population (Table 47.7). Type 1 or "classic" AIH, associated with presence in serum of smooth muscle antibody (SMA) and/or antinuclear antibody (ANA), more commonly affects women and has a pediatric incidence peak between 10 and 20 years of age (100). Type 2 AIH is characterized by liver–kidney microsomal-1 (anti-LKM-1) antibody in serum (101). Children with type 2 AIH tend to be younger, and there is no clear gender predilection. The liver disease at presentation is more likely to be severe or advanced in type 2 AIH; this disorder may even present as fulminant hepatitis (102). A genetic disorder called *autoimmune polyglandular syndrome* or *AIPGS type 1*, is a disease of immune dysregulation. Affected children have some immunodeficiencies, manifest by chronic mucocutaneous candida infections, and some autoimmune endocrinopathies, such as hypothyroidism and adrenal insufficiency. AIH occurs in approximately 25% of cases of AIPGS type 1 (103). A fourth type of AIH seen in childhood is associated with Coombs positive hemolytic anemia, and is histologically characterized by giant cells (104). ANA, SMA, and LKM antibodies are not found in this type. Rarely, "seronegative AIH" is encountered, in which the histologic features of AIH are recognized, no other causes of liver disease are identified, but none of the typical autoantibodies are identified. Minocycline, pemoline, and other drugs sometimes used in children or adolescents have been associated with the development of AIH (105,106,107).

Table 47.7. Types of Autoimmune Hepatitis in Childhood

Autoimmune hepatitis	Autoantibodies	Associated features
Type 1	ANA, ± ASMA	Female preponderance Other autoimmune disorders
Type 2	Anti-LKM-1	Genders equal Younger children Other autoimmune disorders
Seronegative	None	Other autoimmune disorders?
Drug-associated	ANA	Exposure to minocycline or pemoline
After liver transplantation	ANA	Recurrent or de novo after transplantation
With AIPGS-1	None (occasionally anti-LKM)	Autoimmune endocrinopathies Mucocutaneous candidiasis
Giant cell hepatitis with hemolytic anemia	None	Coomb's positive hemolytic anemia
Overlap syndrome	ANA, ± ASMA	PSC

ANA, antinuclear antibody; ASMA, antismooth muscle antibody; Anti-LKM, liver/kidney microsomal antibody; AIPGS-1, autoimmune polyglandular syndrome type 1; PSC, primary sclerosing cholangitis.

Primary Sclerosing Cholangitis

The chronic fibrosing inflammation of the intra- and extrahepatic bile ducts called *sclerosing cholangitis* has been characterized as either primary or secondary. Secondary sclerosing cholangitis may be due to choledocholithiasis, postoperative stricture, toxin-induced bile ductular injury, or in association with other disorders such as acquired immunodeficiency syndrome and Langerhans-cell histiocytosis (108). The cause of PSC (see Chapter 23) is unknown. The association between PSC and inflammatory bowel disease, as well

as the occasional presence in serum of autoantibodies such as antineutrophil cytoplasmic antibody (ANCA) strongly suggests an immunologic cause. Although adults, particularly men, are most commonly affected, PSC has been reported in the adolescent, childhood, and neonatal age-groups, with or without concurrent inflammatory bowel disease (109).

The clinical presentation of PSC is widely variable. Neonates with jaundice resembling that of biliary atresia have been described. Older children can be asymptomatic or present with nonspecific symptoms such as fatigue, vague abdominal pain, and/or pruritus. Cirrhosis and portal hypertension may be the earliest indicators. PSC should be considered in any patient with inflammatory bowel disease, especially ulcerative colitis, who exhibit physical or biochemical evidence of hepatobiliary dysfunction. Although cholangiography may be diagnostic if macroscopic bile ducts are involved, it is not unusual early in childhood to have only microscopic or "small duct" PSC, detected only by examination of liver biopsy specimens. An "overlap syndrome" with features of both AIH and PSC has been noted in children (110), as in adults. A longitudinal cohort study of 52 children with PSC (81% with inflammatory bowel disease) demonstrated that 35% had an overlap syndrome with AIH (111). In this pediatric population, the median survival without liver transplantation was only 12.7 years, indicating that PSC may be a rapidly progressing chronic liver disease in children, as in adults.

Acquired Disorders of the Biliary Tract

Extrahepatic Biliary Atresia

EBA, although not a common disorder, is the most common pediatric indication for liver transplantation. EBA is an inflammatory and progressive destruction of the extrahepatic bile ducts resulting in fibrosis, biliary cirrhosis, and eventual liver failure (6,112). The cause of EBA is unknown, but the most common form is felt to be acquired rather than congenital. Infections, intrauterine and perinatal, metabolic disorders, genetic predisposition, and environmental exposures have been implicated in various studies (6). Current surgical intervention and medical management have dramatically improved the prognosis for this disease since its discovery in 1817. Although EBA was once a universally fatal disease, the developments of the hepatoportoenterostomy (Kasai) procedure and liver transplantation have made it a survivable condition (113).

The most consistent clinical feature of EBA is cholestatic jaundice that appears in the second or third week of life, although some infants may be jaundiced from birth. Hypopigmented or acholic stools are strongly suggestive of this diagnosis. An enlarged and hard liver may be evident at the time of presentation. Congenital anomalies such as splenic malformations, intestinal malrotation, and cardiovascular defects have been associated with the fetal or embryonal form of EBA, as described earlier in this chapter.

The evaluation of an infant suspected of having biliary atresia is essentially the same as that for an infant with neonatal cholestasis (see subsequent text). Frequently, the jaundiced infants will have a mixed hyperbilirubinemia with elevated

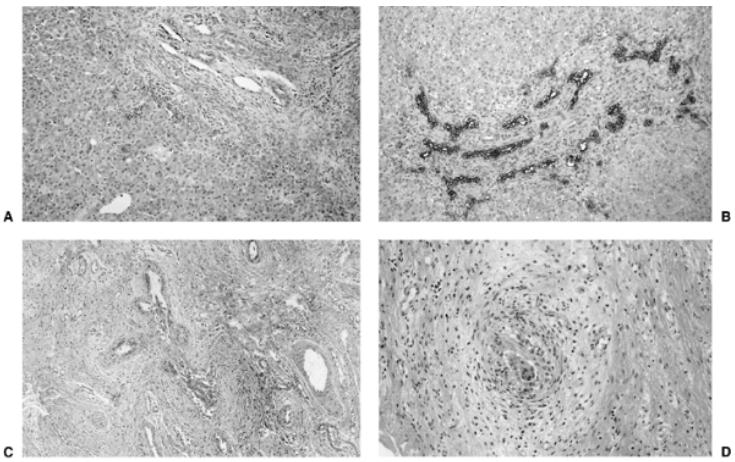
serum AP, γ-glutamyl transpeptidase (GGTP), and aminotransferase values. The absence of a gallbladder on fasting sonography should raise suspicion of EBA, although some affected infants will have a gallbladder. Radionuclide scans are often used to determine biliary patency. Failure of excretion of radioisotope is an indication for liver biopsy and cholangiogram. Bile ductular proliferation and cholestasis are typical histopathologic findings; variable degrees of portal inflammation, occasional giant cell formation, and portal fibrosis are noted (Fig. 47.12A,B). The diagnosis of EBA is confirmed by cholangiogram, usually intraoperative, but sometimes by ERCP. The resected porta hepatis reveals an active destructive and fibrosing extrahepatic cholangitis (Fig. 47.12C, D).

The bile drainage procedure is a hepatoportoenterostomy or "Kasai procedure," after Dr Morio Kasai who developed the surgery in 1968. In this surgery, the scar tissue within the porta hepatis is excised, and a loop of bowel is attached in an attempt to reestablish bile flow from the liver. Recognition of EBA and the timely establishment of biliary drainage through the portoenterostomy are critical. After 3 months of age, the liver injury may be severe enough to make portoenterostomy very unlikely to be of value. Therefore, awareness of EBA and early referral for diagnostic evaluation are essential. In one third of infants treated with portoenterostomy, jaundice never resolves, and hepatic injury progresses rapidly. In approximately another one third, jaundice resolves over several months, but cirrhosis is already established, or develops over the next several years (114). In these children, liver transplantation is the only other treatment option, although supportive measures such as fat-soluble vitamins and choleretic agents are often employed.

Cholelithiasis

Cholelithiasis or gallstones are more common in children than previously thought. Increased awareness and improved detection with techniques such as sonography have played a role in the recognition of gallstone disease as a potentially important pediatric problem (115).

The incidence of cholelithiasis is unknown; this is largely because many infants and children with gallstones are asymptomatic. When symptoms do occur, they are similar to those in adults.



▪ **Figure 47.12** Extrahepatic biliary atresia. **A:** There is marked portal expansion with fibrosis, mixed inflammatory infiltrate, and marked bile ductular proliferation. There is no significant hepatocellular damage. **B:** Immunohistochemical stain for cytokeratin 19 highlights the marked bile ductular proliferation. **C:** Sections of the porta hepatis demonstrate an active destructive and fibrosing cholangitis. **D:** This extrahepatic bile duct remnant shows mixed inflammation, necrosis of the wall, and early periductal fibrosis.

Several conditions have been associated with gallstone formation in children. Hemolytic disease, TPN, short bowel syndrome, CF, and adolescent pregnancy are responsible for most cases. Complications of cholelithiasis include cholecystitis, cholangitis, and perforation; all are more common in adults. Pancreatitis, due to obstruction of the pancreatic duct by a stone or stones in the common bile duct, is the most common complication in children.

Therapy depends on several factors including severity of symptoms, if any, type of gallstone, etiology, and patient factors. As in adults, this may range from expectant management in asymptomatic patients to cholecystectomy for those who have clinically apparent biliary colic, cholangitis, cholecystitis, or an episode of pancreatitis. Endoscopic sphincterotomy with or without stone retrieval is used in the management of bile duct stones. Dissolution therapy using oral bile acids is not effective for pigment stones, which represent most childhood gallstones. Lithotripsy has not been adequately studied in children.

Cholecystitis

Both calculous and acalculous cholecystitis occur in children. Physical examination, laboratory findings, and recommended treatment are similar to those in adults.

Hydrops of the Gallbladder

A massively dilated gallbladder without evidence of gallstones or inflammation is termed *hydrops of the gallbladder* (116). The exact cause of hydrops is unknown, but there is frequently an antecedent or concurrent illness. Acute hydrops of the gallbladder has been reported in association with streptococcal infections, Kawasaki syndrome, and Henoch-Schönlein purpura. Patients vary in age from newborns to adolescents. Symptoms include right upper quadrant abdominal pain and an enlarged, tender, palpable gallbladder. Differentiation between gallbladder hydrops and cholecystitis may be difficult. The best means of diagnosis is ultrasonography, which will reveal a distended and thin-walled gallbladder with no stones, normal

bile ducts, and no evidence of inflammation such as a thickened gallbladder wall. Spontaneous recovery is typical and surgical intervention is usually not necessary.

The Syndrome of Neonatal Hepatitis

Neonatal hepatitis is a syndrome of symptoms, signs, and hepatic histology that includes many types of neonatal liver disease of infectious, genetic, toxic, and metabolic etiologies (117) (Table 47.8). Many of these have already been discussed, and others are discussed in subsequent text because they are specifically pertinent to the differential diagnosis of the syndrome of neonatal hepatitis. The term *neonatal hepatitis* is often used interchangeably with "neonatal cholestasis" because of the prominent conjugated hyperbilirubinemia in most of these disorders. The designation *idiopathic* neonatal hepatitis describes the neonatal liver disease for which no specific etiology can be ascertained. The incidence ranges from 1 in 4,800 to 1 in 9,000 (118). Idiopathic neonatal hepatitis/cholestasis and EBA account for 60% to 70% of all cases of neonatal cholestasis (119).

Table 47.8. Disorders Associated with Neonatal Hepatitis/Cholestasis

ANATOMIC ABNORMALITIES
- Extrahepatic biliary atresia
- Choledochal cyst
- Bile duct stenosis
- Neonatal sclerosing cholangitis
- Spontaneous perforation of common bile duct
- Cholelithiasis
- Masses or neoplastic lesions

IDIOPATHIC NEONATAL HEPATITIS/CHOLESTASIS
- Infections
  - Viral
    - Cytomegalovirus
    - Herpesviruses (simplex, herpes-6 virus)
    - Rubella virus
    - Hepatotropic viruses (A, B, C, D, E)
    - Human immunodeficiency virus
    - Enteroviruses
    - Adenovirus
    - Parvovirus
  - Bacterial
    - Syphilis
    - Bacterial sepsis, urosepsis
    - Listeriosis
    - Tuberculosis
  - Parasitic
    - Toxoplasmosis
- Familial intrahepatic cholestasis syndromes
  - Alagille syndrome
  - Nonsyndromic paucity of the interlobular bile ducts
  - Progressive familial intrahepatic cholestasis (several types)
  - Miscellaneous familial cholestatic syndromes
- Metabolic disorders
  - Disorders of carbohydrate metabolism
    - Galactosemia
    - Hereditary fructose intolerance
    - Glycogen storage disease type IV
    - Carbohydrate-deficient glycoprotein syndrome
  - Disorders of amino acid metabolism
    - Tyrosinemia
  - Disorders of lipid metabolism
    - Wolman's disease
    - Cholesteryl ester storage disease
    - Niemann-Pick disease
  - Disorders of bile acid synthesis
    - 3-β-Hydroxysteroid 5-C_{27} steroid dehydrogenase isomerase deficiency
    - Δ^4-3-oxosteroid 5 β-reductase deficiency
    - Oxysterol 7α-hydroxylase deficiency
  - Peroxisomal disorders
    - Zellweger's syndrome (cerebrohepatorenal syndrome)
  - Mitochondrial hepatopathies
  - Miscellaneous metabolic disorders
    - α_1-Antitrypsin deficiency
    - Cystic fibrosis
    - Neonatal hemochromatosis

CHOLESTASIS ASSOCIATED WITH TOTAL PARENTERAL NUTRITION
- Miscellaneous
  - Vascular disorders of the liver
  - Drug hepatotoxicity
  - Inspissated bile syndrome

Ischemia
Endocrine disorders
Chromosomal disorders
Neonatal lupus erythematosus
Neonatal histiocytosis

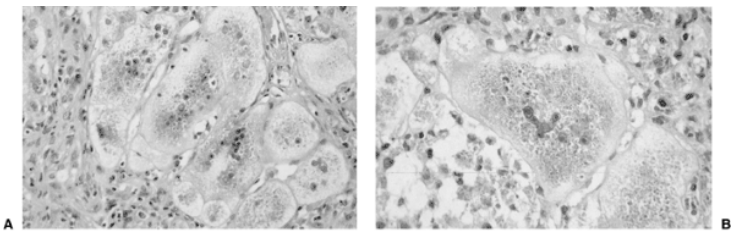
Giant Cell Hepatitis

Giant cell hepatitis is a general term applied when there is extensive or diffuse transformation of hepatocytes into multinucleated giant cells (Fig. 47.13A,B). Giant cell transformation is a common response of the infantile liver to heterogeneous insults. It is not a specific diagnosis and may be associated with a variety of different disorders. The origin and mechanisms of giant cell formation are not clear. They are primarily the result of syncytial fusion of ballooned, degenerated hepatocytes.

Causes of Neonatal Hepatitis/Cholestasis

Congenital infections

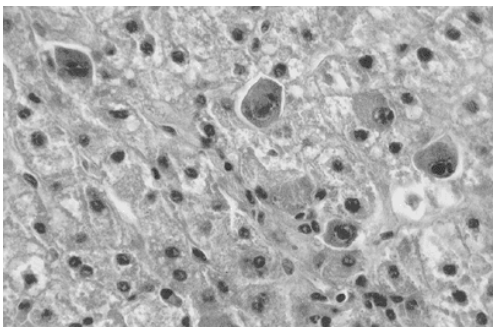
Numerous agents, viral, bacterial, and protozoal, have been implicated in congenital infections associated with hepatitis.



▪ **Figure 47.13** Giant cell hepatitis. **A:** Diffuse giant cell transformation of hepatocytes, cholestasis, and mild lymphocytic infiltrate. **B:** At higher magnification a multinucleated giant hepatocyte reveals cytoplasmic distention with numerous bile granules.

Cytomegalovirus

CMV, a herpesvirus, is the most common cause of congenital infection (120). Clinically apparent disease occurs in only 10% to 15% of infected infants. Signs in the newborn include intrauterine growth retardation, microcephaly, periventricular intracranial calcifications, psychomotor retardation, deafness, and thrombocytopenia. Hepatosplenomegaly with conjugated hyperbilirubinemia and mild elevation of aminotransferase levels are common. Hepatic calcifications may be noted. Hepatic histologic findings include giant cell hepatitis, cholangitis, fibrosis, and persistent extramedullary hematopoiesis. The diagnosis is confirmed by the characteristic viral cytopathic changes (Fig. 47.14) in the bile duct epithelium, hepatocytes, or Kupffer cells. Cytopathic changes may not be present in the liver of neonates; the diagnosis in newborns may be suggested by detection of CMV in a buffy coat preparation, or immunoglobulin M (IgM) antibody to CMV. Treatment of congenital CMV infection includes the use of ganciclovir and CMV immunoglobulin. The liver injury usually resolves if the patient survives the neurologic damage.



▪ **Figure 47.14** Cytomegalovirus (CMV) hepatitis. Characteristic CMV cytopathic changes are seen in several transformed hepatocytes. There is cytomegaly and an intranuclear inclusion surrounded by a halo. Nucleoli appear displaced by the intranuclear viral inclusion.

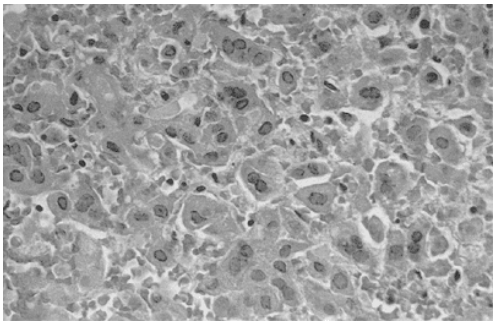
Herpes simplex virus

Herpes simplex virus ([HSV]-1 and -2) may be transmitted vertically, either in utero or perinatally, as the result of ascending infection or contact with cervicovaginal secretions (121). Approximately 90% of congenital herpes infections are due to HSV-2. Primary maternal infection, especially late in gestation, prolonged rupture of membranes, prematurity, and skin trauma such as fetal scalp monitoring are factors that increase the risk of neonatal HSV disease. Perinatal HSV infection is manifested by three syndromes, skin, eye, mouth disease; central nervous system disease; and disseminated HSV disease. Hepatitis is often part of the disseminated disease (122) and it is usually severe with jaundice, hepatomegaly, coagulopathy, and gastrointestinal bleeding. Liver histologic findings include multifocal lytic-type necrosis with characteristic intranuclear inclusions within viable hepatocytes at the periphery of the necrotic areas (Fig. 47.15). Diagnosis is confirmed by isolation of virus from the skin or mucous membranes, immunohistochemistry, and by detection of viral DNA utilizing PCR. Prognosis is ominous without treatment. Early parenteral administration of acyclovir decreases both morbidity and mortality.

Rubella

Congenital infection with rubella occurs transplacentally, and more severe consequences result from infection earlier in gestation. Children with congenital rubella have ophthalmologic, cardiac, and neurologic abnormalities; mental retardation; and sensorineural deafness. Hepatic involvement is frequent (123), and may range from an early presentation with jaundice,

hepatosplenomegaly, and transient cholestasis to a late anicteric hepatitis. Hepatosplenomegaly is a consistent feature and may persist until the end of first year or even later. The diagnosis of neonatal infection is confirmed by isolation of the virus from nasopharyngeal or other body fluids, demonstration of rubella IgM antibody at birth, or a postnatal increase in the infant's IgG titer with stable or decreasing maternal IgG titer. The widespread use of rubella vaccine has resulted in a marked decrease in the incidence of congenital rubella.



▪ **Figure 47.15** Herpes simplex virus hepatitis. Numerous hepatocytes with characteristic cytopathic changes are seen. These include multinucleation, glassy intranuclear inclusions, and margination of chromatin.

Enteroviruses

Transmission of the nonpolio enteroviruses (Coxsackie, echoviruses, and others) may occur during the prenatal, intrapartum, or perinatal period. In 60% of cases there is a history of viral-like syndrome with fever during the last 2 weeks of pregnancy. Infants appear healthy in the first days of life before they develop fever, lethargy, poor feeding, and diarrhea. Hepatitis and jaundice are the most frequent manifestations. In most cases, the course is benign and self-limited. However, progressive hepatic failure with markedly elevated aminotransferase levels, disseminated intravascular coagulation, and massive hepatic necrosis have been reported with infections of Coxsackie group B, echovirus types 6, 11, 14, and 19. Therapy is supportive.

Hepatotropic viruses

HAV is not a common cause of neonatal hepatitis. However, a newborn may become infected if the mother has acute hepatitis during the last 2 weeks of pregnancy. In infants, the infection may be asymptomatic or present as a gastroenteritis-like syndrome. One report documents a premature infant who developed hepatic necrosis during the course of acute hepatitis A. No specific treatment is available. Mothers who are either long-term carriers of hepatitis B or have acute infection during the third trimester of pregnancy may transmit the virus to their infants. Infants born to HBsAg-positive mothers demonstrate serologic or clinical evidence of HBV infection, 1 to 4 months postnatally. Although most perinatally infected neonates are asymptomatic, there are occasional cases of acute icteric hepatitis and even fulminant hepatitis. HCV can be transmitted perinatally. HCV infection in the newborn is usually asymptomatic. Some infected infants, however, may have mild to moderate aminotransferase elevation. Perinatal transmission of hepatitis D is possible, although uncommon (124). Vertical transmission of hepatitis E virus (HEV) has been described. In one report, 6 of 8 babies born to mothers infected with hepatitis E in the third trimester had clinical, serologic, or virologic evidence of HEV infection (125). Most of them had anicteric hepatitis, but one was icteric at birth. Two of them died within 24 hours and one was found to have massive hepatic necrosis.

Human immunodeficiency virus

Hepatosplenomegaly is a common early manifestation of HIV infection in infants, seen in more than 90% of pediatric cases. Cholestatic hepatitis has been reported as the first manifestation of HIV infection as early as 5 months of age (126).

Syphilis

Hepatitis and hepatosplenomegaly are manifestations of early congenital syphilis (127). Jaundice may be the predominant feature. Fulminant hepatitis has been associated with death in a few patients. Characteristic histologic findings are centrilobular mononuclear infiltrates with portal and interstitial fibrosis. Other histologic patterns may be seen, such as neonatal hepatitis with giant cell transformation, nonspecific hepatitis with portal inflammation and portal fibrosis, or even completely normal histology in the presence of heavy infiltration by treponemes. Extramedullary hematopoiesis is seen in both liver and spleen. The treatment is penicillin administered parenterally. Aminotransferase levels may remain elevated for months after treatment. Although treatment may arrest or eradicate the infection, the prognosis depends on the damage done prior to initiation of therapy.

Bacteremia

Bacteremia (128) and urosepsis (129) are well-documented causes of jaundice in the newborn period and jaundice may be the earliest manifestation of sepsis. Hepatomegaly occurs in 40% to 60% of patients, although aminotransferase levels are normal or mildly elevated. The most commonly reported

agent is *E. coli*. Genitourinary evaluation is mandatory although anatomic urinary abnormalities are infrequent. Histologic examination of the liver reveals periportal inflammation, Kupffer cell hyperplasia, moderate cholestasis, and clusters of neutrophils ("minimicroabscesses") within the lobule. The pathogenesis of bacterial sepsis-associated cholestasis is not clear. Hepatocellular damage and enhanced hemolysis causing overload of the immature hepatic function are among the factors considered. Bacterial endotoxin has been implicated in the pathogenesis of hepatocellular injury. It is speculated that the lipid moiety inhibits the activity of Na^+, K^+-ATPase, which interferes with the bile flow at the canalicular level.

Listeria

Direct bacterial infections of the hepatic parenchyma are very uncommon in the newborn infant. The exception is septicemia caused by *Listeria monocytogenes*, in which hepatic manifestations are always present. Listeria is acquired transplacentally or at delivery from infected cervicovaginal secretions. Two histologic forms have been described: Diffuse hepatitis or, more commonly, demarcated areas of necrosis or microabscesses that contain pleomorphic gram-positive bacilli (130).

Toxoplasmosis

Toxoplasma gondii is an obligate intracellular protozoan parasite that can cross the placenta and infect the fetus. Congenital infection occurs primarily as a result of acute maternal infection during pregnancy, by either ingestion of cysts in undercooked meat or direct contact with feces of infected animals such as cats or kittens. The minority of cases result from reactivated disease in pregnant immunosuppressed women, especially those infected with HIV. Severe clinical disease is observed in fetuses infected in the first trimester, whereas those infected in the second and third have mild or subclinical disease at birth. Treatment of acute infection during pregnancy with spiramycin has been shown to decrease the incidence of vertical transmission. Most infants have subclinical disease. Clinical manifestations, when they occur, include microcephaly or hydrocephalus, intracranial calcifications, chorioretinitis, seizures, psychomotor retardation, thrombocytopenia, hepatosplenomegaly, and jaundice. Hepatitis may be the only clinical sign of disease. Hepatic microcalcifications may be detected on plain abdominal radiographs. Liver histology may reveal a nonspecific giant cell hepatitis, but more frequently shows focal areas of necrosis and endothelial cells containing the parasite. The enzyme-linked immunosorbent assay (ELISA) detecting toxoplasma IgM antibody has high sensitivity and specificity. Pyrimethamine and sulfadiazine are synergistic and are the most commonly used drugs in the treatment of infants with documented infection.

Familial intrahepatic cholestasis syndromes

Familial intrahepatic cholestasis syndromes are generally included in the differential diagnosis of the neonatal hepatitis syndrome, because presentation is often in the first months of life. Although they are grouped together arbitrarily because they have similar features, many of these disorders are not completely characterized, and the pathogenetic mechanisms may be unrelated. Some of these disorders have been discussed in the preceding text; these include inborn errors of bile acid metabolism or transport, the three types of PFIC, and Alagille syndrome.

Cholestasis associated with total parenteral nutrition

As discussed in the preceding text, cholestasis and a clinical picture indistinguishable from the neonatal hepatitis syndrome are common in infants who receive TPN.

Miscellaneous causes of neonatal hepatitis syndrome

Vascular disorders of the liver

Vascular disorders are an unusual cause of the neonatal hepatitis syndrome, but they may present with conjugated hyperbilirubinemia, and elevated aminotransferase and AP levels. Two major categories of vascular disorders, hemangiomas and vascular malformations, have been distinguished, on the basis of the cellular biology and natural history of the lesions. Infantile hemangioendotheliomas may also present with jaundice. These vascular tumors are discussed in subsequent text as tumors of the liver in childhood.

Other vascular disorders, such as veno-occlusive disease and Budd-Chiari syndrome are rarely described in neonates, although veno-occlusive disease has been reported in a breast-fed infant whose mother was drinking herbal tea.

Drug hepatotoxicity

Neonatal cholestasis has been reported with chloral hydrate, pancuronium bromide, and erythromycin estolate. Di-2-ethylhexyl phthalate, a plasticizer found in the tubing for extracorporeal membrane oxygenation (ECMO) has been implicated in the pathogenesis of cholestasis seen in infants supported with ECMO. Isolated reports describe infantile hepatitis caused

by halothane and phenobarbital.

Inspissated bile syndrome

Inspissated bile syndrome is a term that was first used to describe conjugated hyperbilirubinemia in fetal erythroblastosis. The liver histology in those infants was similar to that of neonatal hepatitis. Rh-incompatibility and CF are currently the most common causes of inspissated bile syndrome.

Endocrine disorders

Congenital hypothyroidism is associated with neonatal jaundice in approximately 20% of cases. Jaundice may be the presenting symptom and is prolonged if the hypothyroidism is left untreated. Hyperbilirubinemia is usually unconjugated. In contrast, conjugated hyperbilirubinemia has been reported in newborns with hypopituitarism (131,132) and septo-optic dysplasia. Signs of hypopituitarism in the neonatal period include hypoglycemia, jaundice, hepatomegaly, and micropenis in males. Prompt recognition and hormone replacement therapy result in resolution of cholestasis, although delay in treatment may result in progression to significant permanent hepatic injury, and even cirrhosis. It is speculated that cholestasis develops secondary to lack of the trophic hormones, such as growth hormone and cortisol, which modulate bile acid synthesis and flow. Cholestasis has also been reported in infants with primary adrenal insufficiency.

Chromosomal disorders

Both neonatal hepatitis syndrome and EBA have been reported in association with trisomy 17-18 syndrome (trisomy E) and trisomy 21 (Down's syndrome). The mechanism is unknown.

Transient myeloproliferative disorder, an acute leukemia-like disorder that may affect neonates with Down's syndrome, has been associated with diffuse hepatic fibrosis and obstructive jaundice (133). Histologically, there is excessive extramedullary hematopoiesis with myelodysplasia, numerous dysplastic megakaryocytes, hepatocellular damage, hemosiderosis, and pericellular fibrosis (134). The prognosis is poor.

Neonatal lupus erythematosus

Cholestasis and hepatosplenomegaly have been reported in infants with neonatal lupus erythematosus (135). Hepatic histology may demonstrate giant cell transformation, extramedullary hematopoiesis, and ductular proliferation. Paucity of interlobular bile ducts has also been reported. It is speculated that maternal autoantibodies that pass to the fetus transplacentally are involved in the pathogenesis of the liver injury. Cholestasis usually resolves by 9 months.

Tumors of the Hepatobiliary System in Children

Tumors of the hepatobiliary system are uncommon in children and comprise approximately 1% to 4% of all childhood solid tumors. They are most common in young children, and usually found in the right hepatic lobe. Benign lesions such as hemangioma, adenoma, focal nodular hyperplasia (FNH), and mesenchymal hamartoma outnumber the malignant tumors, hepatoblastoma, HCC, and embryonal sarcoma. In general, liver tumors are asymptomatic and are discovered during routine examination as an abdominal mass.

Hemangiomas and hemangioendotheliomas, which arise from vascular endothelium, are the most common benign tumors of the liver in children (136). These lesions are generally asymptomatic but larger or numerous tumors can be associated with hepatomegaly, abdominal pain, bruit, and/or jaundice. Heart failure from arteriovenous shunting within the liver or thrombocytopenia from sequestration of platelets within the tumor, termed *Kasabach-Merritt syndrome*, is sometimes seen. True hemangiomas exhibit rapid growth then slow involution, whereas vascular malformations grow in proportion to the growth of the child and fail to regress. Multiple hepatic hemangiomas are usually associated with cutaneous hemangiomas or diffuse neonatal hemangiomatosis involving the brain, lungs, and gastrointestinal tract. They commonly present within the first 6 months. Hepatic hemangiomas invariably regress late in infancy, but they may be life threatening during the proliferative phase with a mortality of 30% to 80%. Pharmacologic therapy with corticosteroids and IFN-α-2a has been used for symptomatic hepatic hemangiomas, large asymptomatic lesions, and extensive multiple hemangiomas which might cause congestive heart failure (137). The response rate to corticosteroids has ranged from 18% to 70% whereas that to interferon has been reported to be as high as 85%. Hepatic arteriovenous malformations (AVM) are less common than hemangiomas and may have similar clinical presentations. Combined embolization and resection is advised for symptomatic AVMs, because they do not regress with time.

Infantile hemangioendothelioma is the most common liver tumor in the first year of life (138). In 87% of cases it is detected before the age of 6 months. Complete resection of the tumor, if possible, is the treatment of choice.

Hepatic adenomas are usually large, encapsulated solitary masses, most often found in young women. Although liver adenomas have been linked to estrogen use in adults, glycogen storage disease is the more important condition associated with this lesion in children (139). Although these are hepatocellular

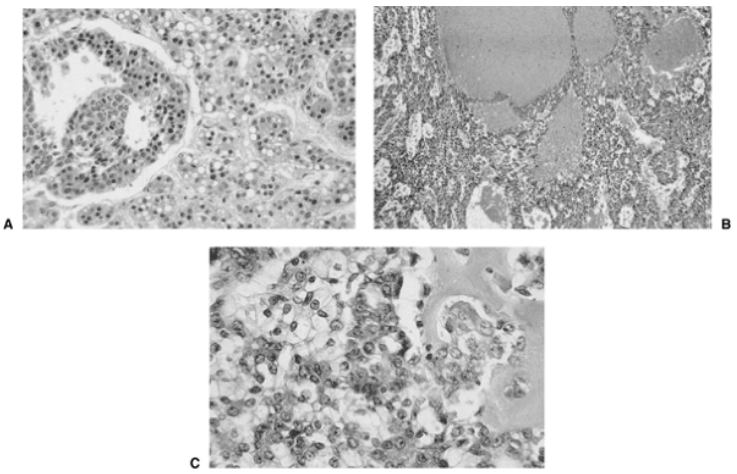
neoplasms, the serum α-fetoprotein level is typically normal. The large nodules seen in galactosemia and tyrosinemia are more accurately designated as nodular hyperplasia, because they occur in the presence of cirrhosis.

FNH is a small, unencapsulated and generally solitary lesion that occurs most commonly in young girls or women but may be noted in either gender and at any age, including infancy and childhood.

Mesenchymal hamartomas (140) are large and multilobulated and cystic tumors. They are asymptomatic, unless the size of the mass is large, causing abdominal pain or jaundice from compression. Histologically, these tumors are comprised of a meshwork of hepatocytes, bile duct epithelia, and mesenchymal cells, intermixed with cystic dilatations within a dense fibrous stroma. The prognosis is usually excellent although malignant transformation into embryonal sarcoma has been reported rarely. Excision is the treatment of choice.

Malignant liver tumors in the pediatric age-group include hepatoblastoma and HCC (140). Hepatoblastomas are single, lobulated, often calcified masses that are usually discovered during infancy. Tumor cells histologically resemble fetal and embryonal hepatocytes and occasionally have mesenchymal components (Fig. 47.16A–C). These tumors may occur in association with Beckwith-Weideman syndrome, hemihypertrophy, and familial adenomatous polyposis. Serum α-fetoprotein levels are almost universally elevated in children with these tumors. Prognosis is fair, with overall survival rates of

approximately 50% depending on the stage of the disease. The lungs and contiguous abdominal spread are common sites for metastasis. Treatment should be aimed at complete resection with pre- and postoperative chemotherapy. Liver transplantation has been successfully used in cases where complete tumor resection cannot be accomplished.



▪ **Figure 47.16** Hepatoblastoma. **A:** Epithelial hepatoblastoma with embryonal (*left*) and fetal (*right*) components. Fetal hepatocytes closely resemble the normal counterparts. **B:** In embryonal areas, peliosis is frequently observed. **C:** Mixed epithelial mesenchymal hepatoblastoma with fetal, embryonal, and mesenchymal components. The mesenchymal component is composed of osteoid-forming malignant cells (*right*).

Childhood HCC arise in association with cirrhosis but can be seen in the absence of underlying liver disease. The incidence is exceedingly high in HT (141). The prognosis is generally poor unless total excision of early tumors is possible. Fibrolamellar HCC occurs in the pediatric age-group, and is not associated with cirrhosis.

The Liver in Cystic Fibrosis

CF, an autosomal recessive multiorgan disorder, is the most common lethal inherited disease affecting the white population, occurring in 1 of 2,000 live births.

The abnormal gene is on the long arm of chromosome 7 and encodes for the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR, a cyclic adenosine monophosphate (cAMP)-dependent chloride channel located in the apical membrane of secretory epithelial cells, promotes efflux of chloride. Several hundred mutations that encode for CFTR proteins with impaired ability to transport chloride ions have been identified. The ubiquity of CFTR throughout the body accounts for the multiorgan involvement that characterizes this disorder. The primary organs affected are the lungs, pancreas, gastrointestinal tract, and liver. In the liver, CFTR is expressed primarily on the apical surface of the biliary epithelium. Largely because of prolonged patient survival and greater awareness, the recognition of liver disease in CF appears to be increasing. Liver disease is not universal; the incidence does not rise progressively but seems to peak during adolescence. However, it is rare for liver disease to have its onset after 20 years of age. Although the reasons for this decline are unclear, two hypotheses have been offered: (a) liver disease increases mortality selectively so that individuals without liver disease are more likely to survive into adult life, or (b) a cohort effect 10 to 15 years earlier than the prevalence studies artificially inflated the likelihood of liver disease, and current adolescent population. Symptomatic liver disease has been documented in 20% of patients with CF and in up to 50% in some series; this can be the presenting or the dominant feature. Pathologic evidence of liver disease, however, is found at autopsy in more than 75% of patients, and is often focal. Cirrhosis complicates CF in 1.4% of patients, with a peak frequency of 2.7% in those 16 to 20 years of age (142). Cirrhosis now accounts for virtually all nonpulmonary causes of death in patients with CF.

The factors that initiate, accentuate, and perpetuate the development of liver disease in patients with CF have not been identified. A 3:1 male preponderance of liver disease is seen in patients with CF (143). Although liver disease is most common in patients with steatorrhea, it may occur in those with intact pancreatic exocrine function (144). Genotype analysis has not revealed a specific mutation in the *CF* gene that correlates with the existence of liver disease. One study has suggested an association with particular histocompatibility antigens, thereby implicating a possible role for altered immune responses in patients with CF and liver disease (145). A more recent study demonstrated an association of a specific polymorphism of glutathione S-transferase with liver disease in patients with CF (146).

The etiology of the hepatobiliary lesions in CF has not been fully elucidated. The description of the gene product cystic fibrosis transmembrane regulator in the apical domain of bile duct epithelial cells (147) suggests that altered ductular secretion results in concentrated viscous bile with subsequent plugging and inflammation.

Several forms of liver disease are seen in patients with CF. Neonatal cholestasis occurs in 2% to 20% of affected infants and may persist for several months. It is generally attributed to viscous bile with sludging. Hepatic steatosis is common, but its cause has not been clearly elucidated. Micronodular cirrhosis is evident in only 2% to 5% of patients and may be multifactorial in etiology. Focal biliary cirrhosis, a lesion virtually unique to CF, is seen in up to 10% to 20% of individuals. Focal biliary cirrhosis begins with accumulation of amorphous material in intrahepatic ducts, which causes focal obstruction, edema, and chronic inflammation. Subsequently, bile duct proliferation and fibrosis evolve into biliary cirrhosis. This lesion occurs most often without signs or symptoms until portal hypertension and its complications ensue. Results of standard biochemical tests may be normal or nearly normal. Patients with CF also have a high incidence of biliary tract disease, including hypoplastic gallbladders, gallstones and/or sludge, common bile duct strictures, common bile duct obstruction from severe pancreatic fibrosis, and a cholangiopathy indistinguishable from PSC.

The optimal method for detection of liver disease in CF has not been established, but several studies have documented the utility of serial ultrasonography in discovery of abnormalities such as steatosis, heterogeneity in echotexture, nodularity, and evidence of portal hypertension, often in the absence of biochemical abnormalities.

Because of pancreatic insufficiency and steatorrhea, most patients with CF should receive supplements of fat-soluble vitamins. However, infants with cholestasis or older patients with severe liver disease may require these supplements in higher dosages because of the additional fat malabsorption associated with low intestinal luminal bile salt concentrations. Because taurine deficiency has been demonstrated in some patients with CF as a result of excessive losses, some authors recommend that supplemental taurine be provided to patients with CF treated with UDCA on the basis of theoretical concern of excessive consumption of this conjugating amino acid induced by large amounts of the unconjugated bile acid. However, in clinical trials, no significant effect of taurine supplementation was documented. Other than the supportive role of nutritional management for chronic liver disease, no other specific dietary therapies are known.

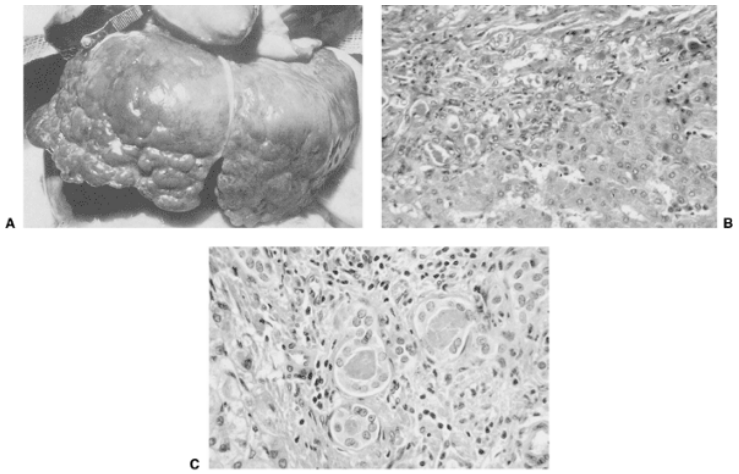
Although the precise pathogenetic effects of the CFTR defect are unknown, it results in the production of thick, tenacious secretions in affected organs, including the hepatobiliary system. Secretion of thick, viscous bile results in impaired bile flow and consequent sludge and potential gallstone formation. Over time, these abnormalities in bile lead to persistent

focal microscopic or macroscopic obstructions in the intrahepatic biliary tree causing a chronic inflammatory infiltrate, bile duct proliferation, and fibrosis with extension and coalescence; the resulting lesion is called *focal biliary cirrhosis* (142) (Fig. 47.17A–C). Focal biliary cirrhosis may be asymptomatic and associated with normal liver biochemical studies, but it appears to be progressive.

Well-established biliary cirrhosis may present as hepatomegaly, splenomegaly, variceal bleeding, or abdominal pain or enlargement. The pathogenesis seems to be an extension of the factors that cause focal disease. The true incidence is undefined. End-stage CF-associated liver disease may be indistinguishable from other forms of severe liver disease.

Other conditions, including reversible focal or diffuse hepatic steatosis, neonatal cholestasis, inspissated bile syndrome, and biliary tract anomalies such as microgallbladder, cystic duct atresia, and bile duct stenosis are also seen. In older patients, a radiographic picture indistinguishable from that of PSC has been reported.

The evaluation of liver disease in children with CF is dictated by the clinical presentation and may include ultrasonography or other imaging modalities such as ERCP. Other causes of liver disease should be excluded. There are several potential, albeit conceptual, approaches to the treatment and ultimate prevention of CF-associated liver disease.



▪ **Figure 47.17** Cystic fibrosis. **A:** Gross appearance of the so-called focal biliary cirrhosi with focal nodularity of the right and left lobes. **B:** Microscopically, portal tracts are expanded with fibrosis, mixed inflammatory infiltrate and bile duct proliferation. Ductal cholestasis is observed. **C:** Bile ducts are distended and contain inspissated pink bile, a feature characteristic of the disease.

Because the presumed underlying pathogenesis focuses on abnormal hyperviscid secretions with bile stasis with the intrahepatic accumulation of hydrophobic, hepatotoxic bile acids, this suggests a rationale for an attempt to decrease the viscosity of bile or to replace or displace hepatotoxic bile acids. UDCA has shown promise in studies of patients with CF-associated liver disease (148). UDCA therapy has been associated with an improvement in biochemical parameters and nutritional status. It is doubtful that the improvement in liver function is solely a function of either an induced choleresis or a change in the hydrophobic/hydrophilic balance of the biliary bile acid pool; UDCA may have some as yet undescribed direct effect at the level of the hepatocyte or biliary epithelial cell. UDCA in pharmacologic concentrations increases intracellular calcium concentrations and stimulates chloride efflux through opening of chloride channels in biliary cells. UDCA may exert a membrane-stabilizing effect on biomembranes exposed to cytotoxic bile acids. However, its long-term benefit, particularly in preventing cirrhosis, remains to be determined.

Management of CF-related liver disease also depends on clinical manifestations and includes assessment of appropriate caloric intake and pancreatic enzyme supplementation. Intervention for variceal bleeding, using sclerotherapy or shunts, has been extensively utilized in patients with CF and cirrhosis. Liver transplantation alone, when possible, may lead to an improvement in both the pulmonary function and nutritional status of CF patients with end-stage liver disease (149). However, the optimal timing of transplantation for an individual patient requires careful thought. Candidacy is influenced by several factors, including lung disease. In the post-transplant period, medication doses must be carefully monitored; patients with CF often need higher doses of drugs because of poor absorption and altered drug metabolism.

Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of disorders that encompasses simple hepatic steatosis and the more serious nonalcoholic steatohepatitis (NASH) that can progress to cirrhosis (see Chapter 39). These increasingly recognized disorders have become one of the most common causes of chronic liver disease in both adults and children. Although the prevalence of NAFLD in childhood is not clear, it has become apparent that it is much more common than originally thought. The major association with NAFLD is obesity, and as the prevalence of obesity in childhood and adolescence increases, fatty liver is recognized with greater frequency. Factors that are associated with progression of liver disease have not been fully determined, but it has become clear that the pathogenesis of NASH is a "two hit" process that includes disturbed lipid homeostasis, resistance to the effects of insulin and subsequent hyperinsulinemia, and local toxic effects of triglyceride on hepatocytes. In adults, steatohepatitis is commonly related to consumption of alcohol, obesity, exposure to hepatotoxic drugs, and diabetes mellitus, but obesity probably plays the most important role in children. Steatohepatitis is usually a clinically silent disease that is commonly discovered when liver test abnormalities are discovered incidentally. However, the clinical spectrum of disease is wide with findings of advanced liver disease occasionally evident at the time of diagnosis. The frequency of progression to cirrhosis is not known.

There appears to be a phenotype of children with NASH that resembles the "typical adult patient": A preteen, asymptomatic obese child (males predominate) who may have hyperlipidemia. In one series (150) of children with steatohepatitis and no evidence of inherited, infectious, autoimmune, endocrinologic, toxicologic, or iatrogenic causes, all 14 patients (10 boys; mean age 13.5 years) were obese (121% to 222% of ideal body weight). Nine patients initially had transient abdominal pain, two had hepatomegaly, and one was identified by incidental laboratory evaluation. Bilirubin levels were normal in all. Five patients had elevated serum cholesterol, and 10 of 12 patients in whom nonfasting triglycerides were measured had elevated levels. All patients had normal nonfasting serum glucose values. All had hepatic imaging studies demonstrating diffuse fatty change.

Although no directly comparative studies have been done, fibrosis may be more common at the time of diagnosis of NAFLD in children and adolescents. One report describes some degree of fibrosis in all 14 children with NASH discovered on a retrospective clinicopathologic review (150). In a study of 17 children, fibrosis was found in 9 (151); one group reports 75% prevalence of fibrosis in 24 children with NASH (152); and another reports fibrosis in 50% of 43 children with biopsy-proven NAFLD (153). It is possible that patient selection for liver biopsy may be more stringent in the pediatric age-group; so far there are no studies that report a large series of biopsies in unselected obese children with ALT or ultrasonographic abnormalities. In any case, instances of advanced liver disease and cirrhosis (152,154) have been described during childhood and adolescence. NAFLD and NASH must be recognized as potential causes of serious liver disease in this population.

Risk factors for progression of liver disease due to NAFLD have been identified in adults. These include age, diabetes mellitus, obesity, and AST:ALT ratio of greater than 1 (155,156,157). Age may be a less consistent risk factor as more cases are being recognized in the pediatric age-group. No risk factors have been confirmed in children or adolescents to date. The higher rate of NAFLD in males may be associated with gender differences in leptin production in obese adolescents.

Laboratory abnormalities in steatohepatitis are mild and nonspecific, and the diagnosis is one of exclusion. Disorders such as Wilson disease, glycogen storage disease, CF, and other metabolic defects may be associated with hepatic steatosis, but usually have other recognizable features. Hepatic steatosis may be suggested by abdominal ultrasonography or computerized tomography but should be confirmed histologically. Liver biopsy specimens will reveal varying degrees of panlobular, macrovesicular steatosis with inflammation and bridging fibrosis. The degree of steatosis, fibrosis, and inflammation does not correlate with symptoms or signs.

Weight loss leads to improvement in or resolution of liver dysfunction, but may be difficult to achieve. The possible contribution of lipid peroxidation causing mitochondrial injury in steatohepatitis might suggest the use of supplemental antioxidants.

Approach to Pediatric Patients with Hepatobiliary Signs or Symptoms

Abnormal "Liver Enzymes"

Persistent aminotransferase abnormalities in children require diagnostic evaluation, because some disorders require specific therapy, whereas others have implications for future health of the patient or family members. The diagnostic approach to abnormal ALT values depends on the age of the patient, exposure history, physical findings, and family history. As in adults, the degree of aminotransferase elevation may not correlate with the amount of liver damage and is often of little prognostic value. Work-up is essentially the same as in adults. Injury or inflammation of cardiac or skeletal muscle also causes increased AST and ALT values. Other causes of elevated aminotransferase values include celiac disease and the presence of a macroenzyme of AST. In the latter, the AST will range from 60 to 1100 IU/L but ALT is normal.

Elevation in GGT is most commonly associated with obstruction or inflammation of bile duct epithelial cells. Although marked elevation of GGT suggests a significant bile duct injury or reduced bile flow, GGT can be paradoxically normal with certain bile acid enzyme or canalicular transport defects such as PFIC-1 and PFIC-2 (see preceding text). Some medications, especially anticonvulsants, induce synthesis of GGT and cause increased serum concentrations. It is not necessary to discontinue these medications for GGT elevations, if other evidence of drug-induced liver injury is absent.

Serum AP activity is less helpful in the assessment of liver disease in children, as it may be elevated for reasons unrelated to hepatic function such as bone injury or growth. Benign elevation of serum AP is an important condition to consider when faced with unexpected elevation in the range of above 1,000 IU/mL. The typical clinical setting is that of an infant or toddler with an apparent viral gastroenteritis in whom AP is measured (158). These very high levels tend to resolve spontaneously over several weeks, although familial persistent elevation of AP is described. In this setting, the serum AP usually comprises primarily the bone isoenzyme. Treatment for this condition is reassurance and avoidance of unneeded, expensive, and invasive tests. Isolated abnormalities in serum AP of the liver isoenzyme are occasionally encountered in infiltrative processes of the liver, namely metastatic disease or granulomatous hepatitis, or as an early indicator of PSC in a child with underlying inflammatory bowel disease.

Hepatomegaly

The liver span will increase as the child grows older but should not exceed 10 cm in childhood. Although the liver span is the

most precise measure (159), palpation of the liver edge is the more conventional method. A liver palpable 2 cm below the right costal margin is considered normal during infancy and 1 cm below the costal margin is acceptable throughout childhood. A liver edge palpable below the xiphoid is atypical and may be another indicator of generalized hepatomegaly. Hepatomegaly may be a transient finding during systemic viral illnesses, such as infectious mononucleosis, but persistent hepatomegaly is an indication for further evaluation. This evaluation would include measurement of aminotransferase and AP values, and some sort of imaging study, such as an ultrasonography. The latter will also detect abnormal echotexture suggestive of fat, fibrosis, or infiltration, as well as space-occupying lesions such as masses or cysts.

Hyperammonemia

The clinical presentation of hyperammonemia is broad and nonspecific. In the newborn period, patients most often present with irritability, fretfulness, and vomiting. Findings on physical examination may likewise be nonspecific with hypotonia, hyperventilation, or apparent respiratory distress. Often, in infants with metabolic disorders, a change in diet or metabolic stress will precipitate an episode of hyperammonemia. A combination of clinical characteristics and plasma amino acid values is used to characterize the patient's underlying condition. Unless the physician suspects a metabolic cause for the clinical symptoms and obtains a measurement of serum ammonia, the infant may succumb within days to progressive acidosis, obtundation, and multiorgan failure.

Hyperammonemia may also occur in older children. As in the newborn period, initial symptoms are variable, but typically include vomiting and/or an altered mental state. Neurologic manifestations are progressive, beginning with increased irritability and confusion, which is followed by combativeness, and only later do patients experience seizures, apnea, or loss of consciousness. Causes of hyperammonemia in children are listed in Table 47.9.

Table 47.9. Causes of Hyperammonemia in Children

Urea cycle disorders
Organic acidemias
Fatty acid oxidation disorders
Disorders of pyruvate metabolism
Transient hyperammonemia of the newborn
Reye's syndrome
Valproic acid hyperammonemia
Congenital absence of the portal vein with portosytemic shunt

Acute Hepatic Failure

Hepatic failure is a well-defined clinical syndrome in adults, comprising the acute onset of encephalopathy, coagulopathy, and evidence of hepatic dysfunction without preexisting liver disease. However, a specific definition is lacking for pediatric patients who may not fulfill all the adult criteria. In particular, encephalopathy in its early stages is difficult to define in infants and children, and when it can be identified may appear late in the course. Therefore, a working definition for children is evidence of liver injury and an uncorrectable coagulopathy in the absence of underlying chronic liver disease. Causes of acute hepatic failure in children are listed by age-group and type of disease in Table 47.10. The diagnostic evaluations for some of these disorders have already been discussed, and for others the principles are the same as in adults. Essential requirements for the management of acute liver failure in children are the same as those in adults.

Table 47.10. Age-Related Differential Diagnosis of Acute Hepatic Failure in the Pediatric Population

Early (<1 wk) perinatal period
Infectious
Herpes simplex virus (HSV)
Echovirus
Parvovirus
Hepatitis B (mutants)
Adenovirus
Metabolic
Neonatal iron storage disease
Respiratory chain defects (disorders of oxidative phosphorylation)
Late perinatal period
Metabolic
Tyrosinemia
Fructosemia[a]
Galactosemia
Zellweger's syndrome
Inborn errors of bile acid biosynthesis (2,35)
α_1-Antitrypsin deficiency
Infectious
EBV
Human immunodeficiency virus
Leptospirosis

HAV
Vascular
Congenital heart disease, myocarditis
Asphyxia
Seizure
Budd-Chiari syndrome
Others
Leukemia
Neuroblastoma
Hepatoblastoma
Hemophagocytic lymphohistiocytosis (familial erythrophagocytic lymphohistiocytosis)
Infants and older children
Infectious (HAV, HBV, HCV, HDV, EBV, CMV, HSV, non-A, non-B, non-C, leptospirosis, togavirus, bacterial sepsis)
Drugs (valproic acid, acetaminophen, pemoline, isoniazid, salicylates)
Toxins (carbon tetrachloride, *Amanita phalloides*, iron overload)
Metabolic (hereditary fructose intolerance, Wilson disease, mitochondrial DNA depletion)
Vascular (shock, myocarditis, sequelae of cardiac surgery, sickle cell anemia)
Other (leukemia, TPN-associated, erythropoietic protoporphyria, autoimmune hepatitis, neuroblastoma, choledochal cyst)
Adolescents
Infectious (HAV, HBV, HCV, HDV, EBV, CMV, HSV, non-A, non-B, non-C, leptospirosis, bacterial sepsis, treponemal infection)
Drugs and toxins (acetaminophen, pemoline, TMP-SMX, drugs of abuse [glue sniffing, cocaine, ecstasy])
Metabolic (Wilson disease, fatty liver of pregnancy)
Neoplastic (lymphoma, leukemia, hepatocellular carcinoma)
Vascular insufficiency (pericarditis, myocarditis, hypoperfusion and hypoxemia)
Miscellaneous disorders (chronic hepatitis, fulminant presentation) (90)
[a]After introduction of sucrose- or fructose-containing feedings.
EBV, Epstein-Barr virus; HAV–HDV, hepatitis A through hepatitis D viruses; CMV, cytomegalovirus; TPN, total parenteral nutrition; TMP-SMX, trimethoprim-sulfamethoxazole.

Complications and Management of Chronic Cholestasis in Children

Some complications are common to all cholestatic disorders: Reduction in intraluminal bile acids resulting in malabsorption of fat and lipid-soluble vitamins; retention of the constituents of bile such as bile acids, bilirubin, and cholesterol, resulting in pruritus and xanthomas; and progressive hepatocellular damage leading to portal hypertension and liver failure. The management of cholestasis is first directed toward specific treatment of the underlying disorder when possible.

Steatorrhea, almost always present in children with severe cholestasis, is a major cause of malnutrition. The combination of long-chain triglyceride malabsorption and inadequate intake may lead to essential fatty acid deficiency. Bile acids are necessary for effective intraluminal long-chain triglyceride absorption as well as for the micellar solubilization of lipid-soluble vitamins (A, D, E, K). In addition, vitamin A and E esters require hydrolysis by pancreatic or intestinal esterases that are bile acid–dependent. Therefore, supplements of preparations with medium-chain triglyceride and fat-soluble vitamins at least two to four times the recommended dietary allowances are necessary. Signs of deficiency as well as toxicity of these vitamins should be monitored periodically by measuring serum vitamin A, E, and 25-hydroxy vitamin D levels, calcium, phosphorus, and prothrombin time. The ratio of serum vitamin E to total serum lipids is more reliable in assessing vitamin E status because elevated lipid levels during cholestasis allow vitamin E to partition into the plasma lipoproteins, artificially raising the serum vitamin E concentration. Prevention and treatment of vitamin E deficiency in chronic cholestasis is best accomplished with a water-soluble formulation of vitamin E, D-α-tocopheryl polyethylene glycol succinate (TPGS), a prodrug in which vitamin E linked to polyethylene glycol is passively absorbed by the intestinal epithelium. This preparation has been shown to increase the absorption of other lipid-soluble chemicals. Hyperlipidemia, xanthomas, and pruritus associated with cholestasis may cause significant morbidity in children, as in adults with chronic cholestasis. Pruritus can be severe and debilitating; it may respond to nonabsorbable anion exchange resins, such as cholestyramine and colestipol, UDCA, phenobarbital, or rifampin as single agents or in combination.

Liver Transplantation in Children

Liver transplantation is reviewed in Part XII of the book. It has become an important therapeutic option for children with chronic or fulminant liver disease, or inborn errors of metabolism (160). Now, with an increasing donor pool, advances in medical and surgical care, and earlier referral to transplantation centers, children are healthier at the time of transplantation and therefore better able to tolerate the surgery with shorter hospital stays.

The indications for orthotopic liver transplantation in children are listed in Table 47.11. Children with metabolic diseases provide a unique challenge. Their illnesses rarely meet the standard criteria for transplantation based on chronic liver disease. In addition, hepatic transplantation in patients with metabolic defects that involve, but are not limited to, the liver (e.g., mitochondrial disorders, some types of GSD) may interrupt the progressive liver disease, but patients often succumb to their condition due to the systemic nature of the diseases.

Survival rates range from 60% to 90%,5 years following liver transplantations in children. Factors that negatively impact survival include age less than 1 year, weight less than 10 kg, care in the intensive care unit (ICU) before transplantation, and a diagnosis of fulminant hepatic failure.

Vascular complications are the most common cause of early postoperative allograft loss and are more likely to occur if the patient weighs less than 10 kg, receives a reduced size graft, develops hypotension during or shortly after the procedure, or has a hypercoagulable state. Hepatic artery thrombosis (HAT) is three to four

times more frequent in children than in adults and occurs most often within the first 30 days following transplantation. Although HAT itself does not lead to hepatic infarction, it commonly causes ischemic injury to the biliary tract. Biliary complications develop in approximately 10% of liver transplant recipients.

Table 47.11. Indications for Liver Transplantation in Children

Biliary tract disorders
Extrahepatic biliary atresia
Alagille syndrome
Caroli syndrome
Genetic/metabolic disorders
α_1-Antitrypsin deficiency
Hereditary tyrosinemia
Cystic fibrosis
Glycogen storage disease
Wilson disease
Neonatal hemochromatosis
Urea cycle defects
Fulminant hepatic failure
"Non-A through E"
Hepatitis B
Drug hepatoxicity
Wilson disease
Autoimmune hepatitis
Chronic hepatitis
Idiopathic neonatal hepatitis
Autoimmune hepatitis
Cirrhosis
Cryptogenic
Total parenteral nutrition-associated cholestasis
Tumor
Hepatoblastoma
Hepatocellular carcinoma

Members of the herpesvirus family cause most of the early and severe viral infections in children after liver transplantation. Patients who are seronegative for CMV and receive an organ from a seropositive donor are at greatest risk for development of either CMV or EBV infection. The clinical manifestations of EBV infection may include a mononucleosis-type syndrome, hepatitis simulating rejection, and extra-nodal lymphoproliferative disease, especially involving the bowel. Post-transplant lymphoproliferative disease (PTLD) is a potentially fatal abnormal proliferation of B-lymphocytes and can occur in any immunosuppressed host. PTLD is seen in up to 15% of all EBV naïve children who are organ transplant recipients. In general, with these considerations, liver transplantation surgery in children should be done at centers that have significant experience with these young patients.

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Chapter 48
Liver Abscesses

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Key Concepts

- A liver abscess is a space-occupying suppurative cavity in the liver resulting from invasion and multiplication of microorganisms, entering directly from an injury, through blood vessels or by the way of bile ducts. The most common forms of liver abscesses are amebic, pyogenic, or mixed in origin.
- The invasive process of *Entamoeba histolytica* in the liver is driven by the motility of the parasites. The parasite relies on a dynamic actomyosin cytoskeleton and on surface adhesion molecules for dissemination in the tissue.
- Emerging new risk factors for amebic liver abscesses include the use of immunosuppressive drugs, sexual lifestyles, human immunodeficiency virus (HIV) infection, traveling to endemic areas, and population migration.
- Risk factors for pyogenic liver abscess (PLA) include aggressive treatment of liver and pancreas malignancies, stent placements, sphincterotomy, embolization, ethanol injection, or radiofrequency ablation.
- Distinguishing between amebic or pyogenic liver abscess is crucial because treatment and prognosis are different. Differential diagnosis relies on clinical history, and laboratory and imaging findings.

A liver abscess is a space-occupying suppurative cavity in the liver resulting from the invasion and multiplication of microorganisms, entering directly from an injury, through the blood vessels or by the way of the bile ducts. The most common forms of liver abscesses are amebic, pyogenic, or mixed in origin. Approximately 60% are solitary and mainly located in the right lobe, as a result of the streaming pattern of portal blood flow secondary to the fact that the right lobe is supplied predominantly by the superior mesenteric vein, and because most of the hepatic volume is in the right lobe. When multiple abscesses are present, pyogenic or mixed abscesses are the most probable types. A detailed clinical history is useful in identifying the risk factors that suggest a possible etiology. Emerging new risk factors include the use of immunosuppressive drugs for neoplastic disease or for organ transplantation, sexual lifestyles, human immunodeficiency virus (HIV) infection, history of traveling to endemic areas, and the population migration phenomena (1).

Amebic Liver Abscess

Epidemiology

Entamoeba histolytica infection is the principal cause of liver abscess in the world, especially in tropical and subtropical regions. It is more prevalent in developing countries; it can be spread from person to person, when polluted water is used to keep vegetables and fruits sold by street vendors fresh. The greatest risk is associated with cyst passers, especially if they are food handlers.

Landmark advances in epidemiology of amebiasis include the recognition that there are two distinct species, *E. histolytica* which is the cause of dysentery, colitis, and liver abscess, and *Entamoeba dispar* which is a nonpathogenic form of ameba (2) and recent technologic advances exploring the genome of the different strains of *E. histolytica* that ultimately may lead to the development of vaccines (3).

Specific probes derived from the analysis of repetitive deoxyribonucleic acid (DNA) sequence in the *Entamoeba* genome analysis have demonstrated possible candidates to distinguish between *E. histolytica* and *E. dispar*, such as transposable elements common in primitive eukaryotes that harbor non–long-terminal-repeat (non-LTR) retrotransposons (also called *long interspersed repetitive elements* [*LINEs*]) (4) or short interspersed repetitive elements known as EhSINE1 (5).

Genotyping of *E. histolytica* has revealed an extensive genetic diversity among *E. histolytica* isolates preventing at present an association of a single genotype with hepatic disease (6). The HM-1 strain of *E. histolytica*, which was isolated from a dysenteric patient in Mexico more than 30 years ago, and those isolated in India and Bangladesh still cause disease in experimental animals and have been used for nearly all immunologic, biochemical, and molecular biologic studies of amebae (7).

Pathogenesis

The invasive process is driven importantly by the parasite's motility. The parasite relies on a dynamic actomyosin cytoskeleton and on surface adhesion molecules for dissemination in the tissues. Myosin II is essential for *E. histolytica* intercellular motility through intestinal cell monolayers and for its motility in the liver, while galactose-binding lectins, mainly galactose/*N*-acetylgalactosamine (Gal/GalNAc), modulate the distribution of trophozoites in the liver and their capacity to migrate in the hepatic tissue (8). Gal/GalNAc acts as a major cell surface antigen that activates target epithelial cells and triggers subsequent disease pathology and parasite survival. Lectin-stimulated cells show an immediate rise in Ca^{+}, which in turn activates cyclic nucleotides and other protein kinases, leading to activation of mitogen activated protein kinase (MAPK) cascade. Activation of MAPK pathway is implicated in events such as apoptosis, proliferation, cytoskeleton rearrangements, and permeability changes (9).

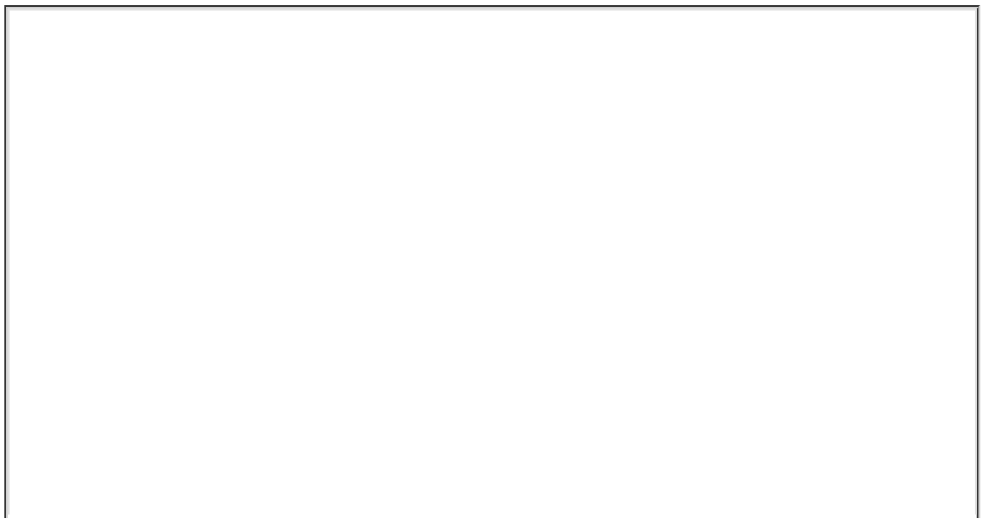
Initial steps in tissue invasion include the release of proteases by trophozoites, which are capable of degrading extracellular matrix components. *E. histolytica* is a cytotoxic effector cell with an extraordinary capacity to lyse the surrounding cells. The inflammatory response (mainly neutrophils and macrophages) initiated by amebic invasion may further contribute to tissue damage by added lysis of parenchymatous cells (10). When ameba is inoculated in the liver significant areas of apoptosis develop within the amebic liver abscess (ALA) (11).

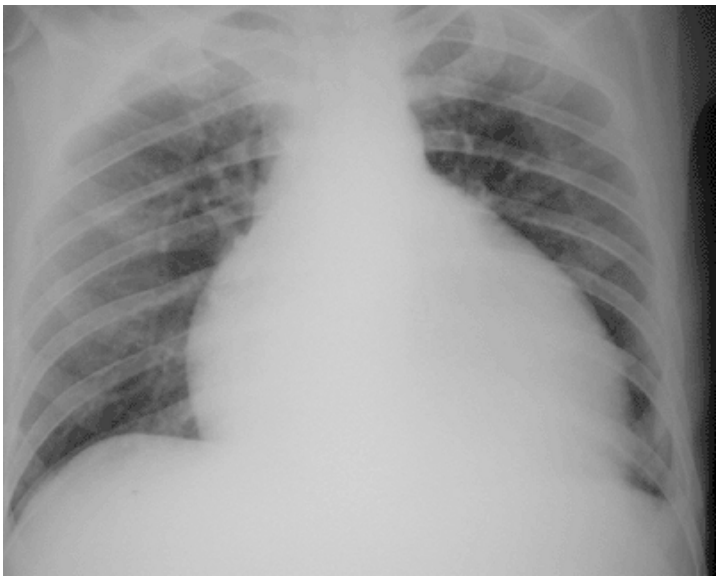
The sequencing of the genome of *E. histolytica* has allowed a reconstruction of its metabolic pathways, many of which are unusual for a eukaryote. On the basis of the genome sequence, it appears that amino acids may play a larger role than previously thought in energy metabolism, with roles in both adenosine triphosphate (ATP) synthesis and nicotinamide adenine dinucleotide (NAD) regeneration (12). *E. histolytica* uses a complex mix of signal transduction systems to sense and interact with the different environments and encounters. The analysis of its genome has revealed almost 270 putative *E. histolytica* protein kinases (13).

Clinical Manifestations

ALA is found more frequently in men between 20 and 40 years of age, but can occur at any age. Gender differences in abscess formation in adults have been related to alcohol consumption. Identification of risk factors including a history of travel to or residency in endemic areas several weeks or even months before, must be identified. Almost always the patients present with a constant, dull, and intense right upper quadrant abdominal pain that exacerbates with movement and frequently radiates to the scapular region and right shoulder. Patients have fever between 38°C and 40°C, chills, and sweating. Very frequently they give a history of malaise and nausea in the previous 2 weeks and moderate weight loss. Some patients have cough and chest pain. Most of them do not have coexistent dysentery, although a past history of diarrhea or dysentery is present in approximately 50% of cases. At physical examination, the patient appears pale and wasted, with painful hepatomegaly, point tenderness over the liver, below the ribs, or in the intercostal spaces. When the abscess is located in the left lobe, the patient may have epigastric tenderness. Ventilation in the right lung is frequently restricted, respiratory sounds are reduced, and jaundice is infrequent. Alarm signs include abdominal rebound tenderness, guarding, absence of bowel sounds, and pleural or pericardial rub. The abscess may extend to the peritoneum, abdominal organs, great vessels, pericardium (Fig. 48.1), pleura, bronchial tree, and lungs (14,15). Common differential diagnoses include pyogenic liver abscess (PLA), primary hepatic carcinoma, liver metastases (Fig. 48.2), or hydatid cysts. The clinical presentation of an ALA among high-risk groups such as patients with HIV or other immunosuppressed individuals is similar to that described for the remaining patients (16). Neonates present with

nonspecific clinical and laboratory findings mimicking fulminant neonatal sepsis (17).

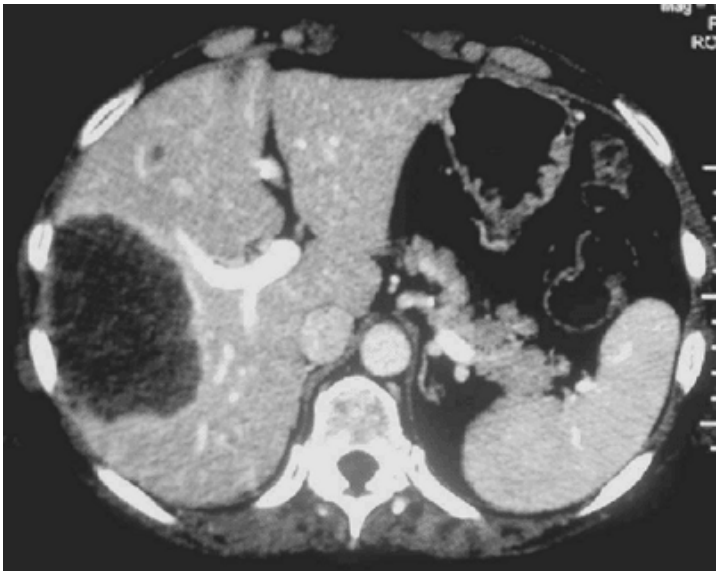




▪ **Figure 48.1** Amebic liver abscess ruptured into the pericardium.

Diagnosis

Leukocytosis (>15 × 10^9 cells/L), with neutrophilia, increased erythrocyte sedimentation rate, slight anemia, and elevated alkaline phosphatase levels are common. Serum antibodies to *E. histolytica* are detected in more than 90% of patients. An indirect hemagglutination (IHA) with a cutoff value of 1:512 is considered diagnostic. The enzyme immunoassay (EIA) with a sensitivity of 99% and specificity greater than 90% is also commonly used. In those patients in whom an aspirate is obtained, either for diagnostic or therapeutic purposes, the material should also be sent for Gram's staining and culture (18).



▪ **Figure 48.2** Liver metastases of colorectal cancer. No specific

characteristics can differentiate it from primary neoplasia, or pyogenic or amebic liver abscess.

Imaging studies are very important in the workup of patients with suspected ALA and have reduced the delay in diagnosis. Ultrasonography is the initial screening choice. The abscess appears as an hypoechoic round or oval lesion with well-defined margins. More advanced imaging techniques such as tomography or magnetic resonance (MR) are indicated for differential diagnosis. Chest x-ray may reveal elevation of the right diaphragm, atelectasis, and pleural effusion (19).

Therapy

The drug of choice is metronidazole at an oral dose of 1g twice daily for 10 to 15 days in adults and 30 to 50 mg/kg daily for 10 days divided in three doses in children; when given intravenously the dosage is 500 mg every 6 hours for adults and 7.5 mg/kg every 6 hours for children for 10 days. Other nitroimidazoles include tinidazole or ornidazole at a dose of 2 g orally daily for 10 days. Secondary drugs include chloroquine 1 g/day for 2 days orally followed by 500 mg/day for 2 to 3 weeks. In children the dose is 15 mg/kg PO daily for 2 to 5 days followed by 5 mg/kg daily for 2 weeks. Percutaneous drainage may be necessary in nonresponders to antiameba therapy to rule out a pyogenic abscess or when less than 1 cm of rim liver tissue remains around a liquefied abscess.

Vaccination

Studies in experimental animals, using *E. histolytica* galactose-and *N*-acetylgalactosamine-inhibitable surface lectin either for systemic application or oral administration are ongoing. Research has focused on how to fuse surface lectin to the B subunit of cholera toxin, attenuated salmonella, or *Yersinia enterocolitica* for antigen delivery (20).

Pyogenic Liver Abscess

Epidemiology

The incidence of PLA is 0.007% to 2.2% of hospital admissions, 11 per 1 million in general population, and between 0.29% and 1.47% in autopsy series. PLA varies among different geographic regions influenced by the local prevalence of bacterial, parasitic, and helmintic infections, age of the population, and the presence of chronic debilitating diseases. Benign or malignant biliary tract disease, diverticulitis, and Crohn’s disease are the most common predisposing factors. The frequency of PLA has increased as a complication of more aggressive treatment of liver

or pancreatic malignancies—stent placement, sphincterotomy, embolization, ethanol injection, or radiofrequency ablation. In the past, PLA was primarily a complication of ruptured appendix (21). Accordingly, the age of presentation has moved forward from the second and third decades of life to the sixth and seventh (21,22). Advances in imaging techniques and new antibiotics, have decreased the morbidity and mortality of PLA (22,23).

Pathogenesis

Abscess formation, a host defense strategy to contain the spread of infection, is promoted by a combination of factors that impair phagocytosis and the clearance of microorganisms. Neutrophils and platelets attached to the endothelial surface, and endotoxin produced by gram-negative bacteria contribute to tissue injury by releasing proinflammatory cytokines and reactive oxygen species. When bacteria reach the liver, endotoxin stimulates the proliferation of Kupffer cells that engorge, and produce toxic mediators that modulate microvascular response. After adhesion, diapedesis through cell junctions follows. The inflammation thus produced causes obstruction of the sinusoidal lumen and secondary obstruction of the blood flow. These phenomena inhibit sodium and potassium ATP activity, impair the generation of energy for bile excretion, and promote biliary stasis. The sinusoidal diameter is reduced and hence the velocity of the blood flow decreases; as the number of obstructed sinusoids increases and hydrostatic pressure rises, hepatic ischemia develops (24).

The source of infection determines to a certain degree the localization and number of abscesses. If infection reaches the liver through the portal system, several abscesses may develop, mostly confined to the right lobe. The left lobe is usually involved in septic thrombosis of the portal vein. When bacteria reach the liver through arterial circulation, several small abscesses develop, equally distributed in both lobes. Forty percent of patients with PLA have multiple liver abscesses (25,26).

Clinical Manifestations

An early clinical diagnosis requires a high index of suspicion: Fever, malaise, right upper quadrant abdominal pain, nausea, and vomiting for more than 2 weeks are the most common presentations (21,27). Abdominal pain in patients with PLA is similar to that found in patients with ALA (25). Other symptoms, anorexia, jaundice, and painful hepatomegaly are less prevalent in PLA as compared to ALA (26,28,29,30). Jaundice predicts a complicated clinical course but has no impact on mortality. Approximately 60% have an underlying debilitating condition or have had a recent interventional procedure (e.g., biliary stent placement, ethanol injection). PLA should be suspected in elderly patients, in those taking steroids, or in patients with right-sided pulmonary abnormalities of unknown origin (30,31).

Diagnosis

When PLA is diagnosed, prognostic factors associated with increased mortality include low albumin, anemia, high blood urea nitrogen (BUN) and creatinine, prolonged prothrombin time, polymicrobial infection, pleural effusion, high acute physiological assessment and chronic health evaluation (APACHE) II score, disseminated intravascular coagulation, and septic shock. Multiple abscesses carry a high mortality risk independent of other risk factors (23). Regarding PLA in patients with cancer, morphology and topography are not different from noncancer patients (25).

Distinguishing Characteristics

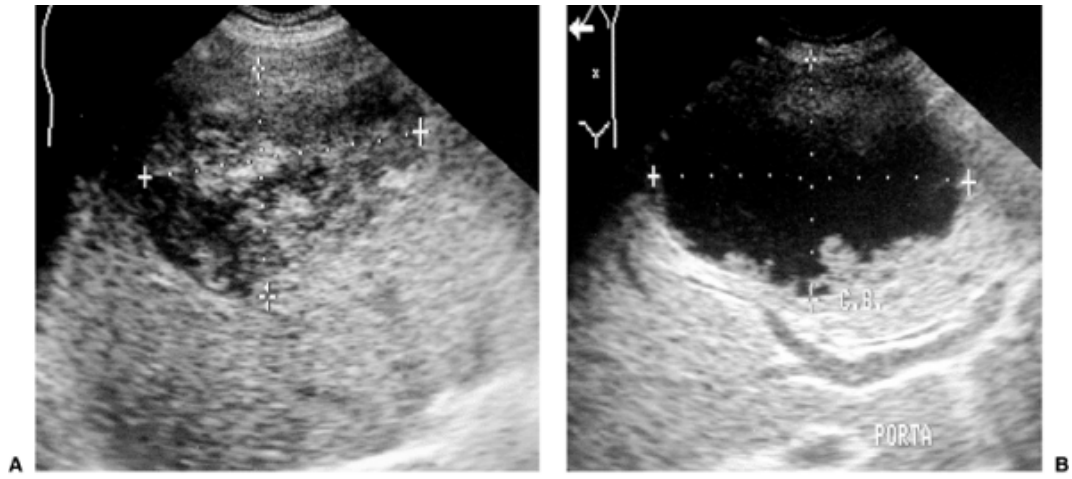
Distinguishing PLA from ALA is crucial because treatment and prognosis are different. Detection of PLA relies on laboratory and imaging findings (Table 48.1). Abdominal ultrasonography and computed tomography (CT) scan have

sensitivities greater than 95% in detecting abscess formation (23,27). PLA and ALA have similar imaging characteristics (Figure 48.3) (28).

Patients with PLA are older than those with ALA, and more likely to have debilitating diseases; this might explain the lower concentration of albumin found in patients with PLA. Abnormal chest findings are more prevalent in patients with PLA (28).

Table 48.1. Diagnostic Differences Between Amebic Liver Abscess and Pyogenic Liver Abscess

Amebic liver abscess	Pyogenic liver abscess
Leukocytosis >15 × 10^9	Leukocytosis >15 × 10^9
Serum antibodies to *E. histolytica* >1:512	Serum antibodies to *E. histolytica* negative
Aspirated pus shows no microorganisms	Aspirated pus shows bacteria
Jaundice uncommon (<8% of patients)	Jaundice common in presentation
Abnormal chest finding in physical examination uncommon	Abnormal chest findings in physical examination common
Younger patients (usually 20 to 40 yr)	Older patients (usually 60 to 70 yr)
Previously healthy	Presence of debilitating disease (cancer)



▪ **Figure 48.3** Pyogenic liver abscess. **A:** Initial ultrasound showing debris inside the abscess cavity. **B:** Liquefied pus inside the abscess.

Laboratory Findings and Microbiology

These data include leukocytosis, anemia, elevated alkaline phosphatase, positive C-reactive protein, and negative *E. histolytica* antibodies. Other findings are increased total bilirubin, low albumin level, and prolonged prothrombin time.

Microbiology studies are the gold standard in establishing the diagnosis of PLA. Specimens of pus and blood should be obtained for culture under strict anaerobic and microaerophilic techniques. Gram's stain guides the selection of the antibiotic regime, pending the result of culture. One third of PLAs are caused by aerobic bacteria, one third by anaerobes, and one third by a mixture of aerobic and anaerobic bacteria. Enteric gram-negative rods are the most frequent isolates in either blood or pus aspirated from the abscess. In children, *Staphylococcus aureus* is the most frequent organism. Only 50% of anaerobic PLA are diagnosed by culture, because of defective sampling or suboptimal laboratory techniques, but this should not delay the initiation of antibiotics. A combination of antibiotics against different microorganisms is the treatment of choice (21).

Bacteriology of PLA is evolving, in some areas of the world specific bacteria have been isolated. *Klebsiella pneumoniae* is being found in PLA in the Asian population in America, suggesting either an epidemiologic transition or the continued prevalence of an endemic infection in a specific ethnic group (22,25,26,28). Resistant bacteria and fungi are opportunistic infections in patients with debilitating conditions (31). Isolation of fungi is frequent in patients with manipulated biliary stents or with intermittent cholangitis treated with broad-spectrum antibiotics. Immunocompromised individuals can develop PLA with other microorganisms such as *Salmonella* (30).

Imaging

Ultrasonography is the screening test of choice. CT scan can detect collections as small as 0.5 cm in diameter and allows therapeutic interventions (e.g., needle aspiration/drainage). The presence of aggregates of multiple small abscesses

suggests the coalescence into a larger abscess (cluster sign) and indicates a PLA (21). MR using serial gadolinium-enhanced gradient-echo images can help in differentiating PLA from other focal lesions (32). MR cholangiogram can be useful in planning possible resectional therapy. All of these modalities are able to demonstrate loculation and consistency of the contained pus to make therapeutic decisions.

Therapy

Antibiotic therapy choices involve combining broad-spectrum antibiotics: Third-generation cephalosporin plus clindamycin or metronidazole; broad-spectrum penicillin plus aminoglycosides; and second-generation cephalosporin plus aminoglycosides. Treatment should be started immediately after specimens have been obtained for culture without waiting for definitive results. Imipenem, aztreonam, piperacillin, tazobactam, ticarcillin, clavulanate, and quinolones are active against almost all aerobic gram-negative bacilli (21). Antibiotics should be given before, during, and after drainage and surgical procedures. Parenteral therapy for 2 to 3 weeks followed by oral antibiotics for 4 to 6 weeks is recommended. For a solitary abscess less than 5 cm in diameter, confirmed by aspirate and

with available antimicrobial sensitivity, resolution can be achieved with antibiotics alone (33,34). For PLA greater than 5 cm with thick and viscous pus, or for those large multiloculated abscesses, surgical drainage may be necessary (35).

Aspiration and Drainage

Under imaging guidance, PLA can be aspirated and drained. Drainage is most effective when well liquefied pus is completely evacuated. If the abscess is not well liquefied or has a thick wall, it is impossible to remove the pus completely. In such cases, most of the drainable pus is removed by needle aspiration, after which, antibiotic therapy is necessary to treat the residual abscess. Needle aspiration should be performed with an 18-gauge fine-walled needle. In multiloculated abscess, the needle tip should be inserted into the various loculi to evacuate pus as completely as possible. Percutaneous needle aspiration is considered unsuccessful when patients fail to improve clinically or radiologically after the second aspiration. Factors affecting drainage include accessibility, number, and size of the abscesses as well as the patient's general condition. Abscesses most accessible to percutaneous drainage are the posterior right lobe deep-seated lesions, those that adhere to the abdominal wall, and peripheral abscesses of the right lobe (36).

Surgery

Surgical therapy is necessary for multiple macroscopic or multiloculated abscesses, or for those in the left lobe, after percutaneous drainage failure. Surgical drainage may be required in the presence of ascites or renal failure, evidence of clinical deterioration, persistent jaundice, or concomitant steroid therapy; or when abscesses are not accessible to radiologic manipulation; and in the case of a ruptured abscess (34,35).

Endoscopic Drainage

In patients with stones or strictures of the bile duct and abscess formation in

continuity with the biliary system, endoscopic therapy provides biliary drainage, promoting abscess drainage (37).

Annotated References

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Features distinguishing amoebic from pyogenic liver abscess: a review of 577 adult cases. *Trop Med Int Health* 2004;9:718–723.

This paper analyses the largest population of patients with pyogenic liver abscess since 1938. It is a good analysis of the available current therapies.

Oral vaccination with recombinant yersinia Enterocolitica expressing hybrid type III proteins protects gerbils from amebic liver abscess *Infect Immun* 2004;72:7318–7321.

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Chapter 49
Parasitic Diseases

Michael A. Dunn

Key Concepts

- Liver parasites span a wide range of complexity, from intracellular protozoa to visible multicellular helminths with highly evolved life cycles. Different species mature and reproduce within hepatocytes, reticuloendothelial cells, the portal venous system, and the bile ducts.
- Well-adapted parasites cause minimal acute injury to the host organ as they generate enormous numbers of progeny that pass into the blood or bile with the potential to infect other hosts. Examples include the malaria parasites, the schistosome worms, and the bile duct flukes.
- When a parasite enters a species or an organ to which it is poorly adapted, acute or severe injury is likely: *Echinococcus* tapeworms, well adapted to canine-herbivore or canine-rodent life cycles, cause severe cystic liver disease in accidental human hosts. The protozoan *Entamoeba histolytica* and the roundworm *Ascaris*, both well suited to the human intestinal lumen, cause acute injury when they invade the liver parenchyma or bile ducts, respectively.
- Successful parasites have evolved to evade or to accommodate the effects of the defenses and immunologic responses of healthy hosts. Hosts with abnormal or compromised responses are at risk for severe disease manifestations, such as the reactivation of subclinical *Leishmania* infection with development of advanced visceral leishmaniasis in human immunodeficiency virus (HIV)-infected persons.

Protozoal Diseases

Malaria, one of the world's most serious and widespread infectious diseases, is intimately involved with the liver during its pre-erythrocytic and exoerythrocytic stages of development. Visceral leishmaniasis is a cause of severe debility and hepatosplenomegaly in the tropics and a growing concern for immunosuppressed persons in temperate climates.

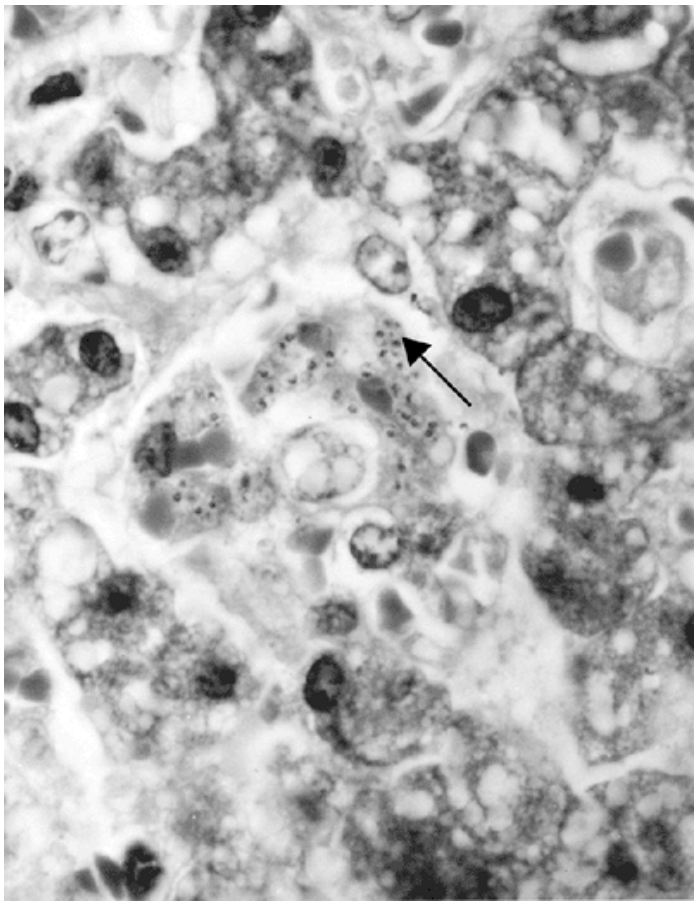
In addition to these obligatory intracellular parasites, *Entamoeba histolytica*, an extracellular protozoan cause of liver abscess, is considered in Chapter 48.

Malaria

Malaria, the world's most prevalent fatal parasitic disease, is caused in humans by intracellular protozoa of four species, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. All are transmitted by mosquito bite, and involve the entry of mosquito-borne sporozoites into hepatocytes, where a pre-

erythrocytic stage of the parasite multiplies and is subsequently released to invade erythrocytes. Malaria continues to kill 2 million persons a year and frustrate eradication efforts because of the parasite's capacity to develop drug resistance. There is, therefore, great interest in defining the events within the liver that may account for complete protection of animals with a vaccine directed against genetically attenuated sporozoites (1). Protection of immunized animals appears to depend both on antibody-mediated recognition of a sporozoite surface protein that binds to liver extracellular matrix proteoglycans, and cell-mediated responses to attenuated

parasites that enter hepatocytes but fail to multiply (2).



▪ **Figure 49.1** Visceral leishmaniasis. Dot-like organisms (*arrow*) are present in several hypertrophied Kupffer cells (hematoxylin-eosin, ×1,000). (Armed Forces Institute of Pathology [AFIP] Negative 87-5647.)

The cyclical fevers, hemolysis, vascular stasis, shock, and multiple organ failure of severe malaria are the clinical end results of synchronized multiplication and release of the parasite's erythrocytic stage. Kupffer cells take up released hemoglobin degradation products known as malarial pigment, which appears as dark cytoplasmic granules in liver specimens from persons with a history of malaria. Humans with intact host defenses normally recover from acute episodes of malaria. The highest risk for severe illness and death is with *P. falciparum* infection. Falciparum malaria often produces clinical and laboratory evidence of multiple organ dysfunction. In two reviews, 60% of 106 patients (3) and 20% of 91 patients (4) showed modest

elevations in serum bilirubin, aspartate aminotransferase, or alkaline phosphatase levels. Severe liver injury in malaria has only infrequently been reported in patients with heavy *P. falciparum* infections, commonly associated with acute renal failure and encephalopathy. In a report from India, seven such patients presented with the acute onset of jaundice, asterixis or impaired sensorium, bleeding with prolonged prothrombin and partial thromboplastin times, and aminotransferase elevations at fourfold the normal (5). *P. falciparum* infection was evident in their blood smears. The three survivors responded to intravenous quinine and supportive care that included lactulose and bowel cleansing. One of the four patients who died had submassive hepatic necrosis; focal steatonecrosis was present in postmortem liver specimens from the other three. As with earlier similar reports, it is unclear to what extent liver injury contributed to morbidity in these patients. The key message remains, however, that a clinical presentation suggesting acute hepatic failure in persons at risk for malaria infection should prompt consideration of this readily diagnosed and treatable illness.

Leishmaniasis

Visceral leishmaniasis, or kala-azar, is an infection of the reticuloendothelial cells of the liver, spleen, bone marrow, and other organs with an intracellular protozoan parasite, *Leishmania*. Common throughout the tropics, visceral leishmaniasis has been increasingly recognized elsewhere as a potential problem for immunosuppressed persons with human immunodeficiency virus (HIV) disease or after organ transplantation (6). As an experimental disease model, leishmanial infection provides an opportunity to study liver inflammation and fibrosis with the same methods that have advanced our knowledge of these important processes in other diseases, such as hepatic schistosomiasis.

Infections with different species of *Leishmania* cause visceral, cutaneous, and mucocutaneous patterns of disease. Visceral leishmaniasis, involving the liver, normally results from infection of children and young adults with *Leishmania donovani*. In the Indian subcontinent, the parasite is transmitted by sandflies that have bitten infected humans. Elsewhere—in South America, southern Europe, Africa, the Middle East, and China—*L. donovani* transmission to humans by sandflies is primarily enzootic, involving canine and rodent reservoir hosts. Visceral involvement with *Leishmania tropica*, a species that normally causes cutaneous disease, was described in eight American veterans of the 1991 Persian Gulf War (7), and two more recent cases of visceral disease due to *L. donovani* were reported in American soldiers exposed to infection in Afghanistan (8). Other than infection from the bite of sandflies in endemic areas, clinical studies suggest that *Leishmania* can be transmitted by blood transfusion or needles shared for drug abuse, by sexual contact, or by transplantation of infected organs (9,10,11,12).

After bloodstream infection and uptake of the parasite by reticuloendothelial cells, its amastygote stage, shown in Figure 49.1, multiplies within Kupffer cells and macrophages, infects new cells, and triggers cellular and humoral host responses. Most immunocompetent persons respond to infection with a successful T helper cell 1 (Th) 1-type cell-mediated defense that prevents clinical disease and suppresses, but may not

eliminate, the infection (13). Such a cellular response, akin to that seen in tuberculoid leprosy or successfully contained initial *Mycobacterium tuberculosis* infection, involves the same T4 cells and cytokines—interferon-γ, interleukin (IL)-2, and IL-12—that are critical for dealing with other intracellular organisms. The potential importance of inducible synthesis of nitric oxide as an effective mechanism for Th 1–mediated killing of the parasite was suggested by a report of high

susceptibility of Syrian hamsters, which are deficient in nitric oxide generation, to severe visceral disease after *L. donovani* infection, despite their ability to mount a prominent and otherwise complete Th 1 cytokine response (14). Humoral antibody responses, also regularly present, do not appear to modify the course of leishmanial infection, nor do the Th 2–type cellular responses seen in persons who develop clinically severe disease.

Pathologic examination of liver specimens shows findings that parallel the predominant host response: In persons with minimal disease and few parasites visible in liver specimens, epithelioid granulomas, including fibrin-ring granulomas similar to those described in Q fever, may be present (15). Numerous parasites multiplying within activated Kupffer cells and macrophages, appearance of myofibroblasts, deposition of intralobular collagen, and effacement of the space of Disse with connective tissue all accompany an ineffective response in persons with overt disease (16,17). A pattern of severe intralobular liver fibrosis as a predominant finding was described by Rogers (18) in 1908 as a "peculiar cirrhosis" in Indian patients with visceral leishmaniasis. So-called Rogers' cirrhosis, however, shows normal liver architecture and no regenerative nodules.

The major clinical manifestations of visceral leishmaniasis include fever, weight loss, hepatomegaly, splenomegaly, lymphadenopathy, pancytopenia, and hypergammaglobulinemia. All organs with reticuloendothelial cells may be involved, including the entire gastrointestinal tract. Laboratory abnormalities may include modest elevations in serum aminotransferase and alkaline phosphatase levels and depressed albumin levels, as well as skin test anergy to common delayed hypersensitivity antigens. Although hepatic and splenic enlargement from cellular infiltration may be truly massive and intralobular liver fibrosis may be pronounced, overt ascites is uncommon, and the very rare occurrence of either hepatocellular failure (19) or of clinically evident portal hypertension suggests another etiology. Persons with advanced disease are at risk of death from intercurrent infections or severe malnutrition. Conversely, malnutrition, immunosuppressive therapy, or an immunosuppressive disease such as HIV infection can precipitate the development of overt visceral leishmaniasis in previously healthy persons with latent infections acquired as long as 20 years earlier (20,21).

Severe HIV-associated visceral leishmaniasis has been described in reports from Spain, a *Leishmania* endemic area, as well as from nonendemic countries such as France and Germany (11,21,22,23). Visceral leishmaniasis should be sought as a treatable cause of fever, hepatosplenomegaly, and rapid deterioration in HIV-infected persons with even a remote positive travel history or risk factors for non–sandfly-transmitted blood-borne infection. Intracellular parasites may be seen in liver or intestinal biopsy specimens obtained during the evaluation of HIV-infected persons for persistent fever or diarrhea (24). Most such patients have CD4 cell counts less than 400/mm^3, consistent with the importance of an intact Th 1 cellular response for dealing with leishmanial infection. This relationship supports the suggestion that visceral leishmaniasis in HIV-infected persons should be considered as an acquired immunodeficiency syndrome (AIDS)-defining illness (11,23). Most HIV-positive patients with visceral leishmaniasis respond well to antimonial therapy; however, relapse after cessation of treatment is common, so that long-term suppressive therapy with fluconazole, ketoconazole, or pentamidine is often used (23).

Visceral leishmaniasis may also become manifest in immunosuppressed liver, heart and kidney transplant recipients (12,25); in one report, the donor liver was considered the likely source of parasite infection (12). Reduction of the immunosuppressive regimen to the minimum needed to support graft function, combined with initial antimonial and long-term suppressive antiparasitic therapy, is

the recommended management.

The diagnostic procedure for visceral leishmaniasis with the highest accuracy, close to 100%, is the examination and culture of a needle splenic aspirate. In nonendemic areas where the experience levels to safely perform splenic aspiration are lacking, examination and culture of bone marrow and liver biopsy specimens are more often performed. Either method provides a 50% to 80% yield. Polymerase chain reaction–based detection of leishmanial DNA in peripheral blood has now been reported to match the diagnostic accuracy of bone marrow aspiration (26,27). The mainstays of therapy for visceral leishmaniasis are pentavalent antimonial compounds; in the United States, the Centers for Disease Control drug service provides sodium stibogluconate as an investigational drug. Its use by daily intramuscular or intravenous administration for 3 weeks or longer is safe and generally effective. Second-line antileishmanials include pentamidine, amphotericin B, allopurinol, and ketoconazole and related azole compounds. A new drug-delivery strategy of incorporating amphotericin B into liposomes or lipid complexes appears to greatly enhance its efficacy: 5-day courses of lipid-complexed amphotericin B at total doses of 5 to 15 mg/kg were

remarkably effective in patients who had not responded or relapsed after antimonial therapy (28).

Although the clinical manifestations of visceral leishmaniasis resolve with antiparasitic therapy, it remains unclear, especially in persons with underlying immunologic deficits, whether the infection is ever truly eliminated or only suppressed below detectable limits (29). The successful use of highly active antiretroviral therapy (HAART) in HIV-infected patients, for example, is thought to be responsible for recent major decreases in the incidence of clinically overt visceral leishmaniasis in France and Spain, although HIV-infected persons with inapparent leishmanial infection remain at lifelong risk for the occurrence of clinical illness (30,31).

Visceral leishmaniasis has attracted investigative interest because of its association with severe intralobular liver fibrosis, which appears to be fully reversible after treatment of the infection (16). When experimental animal systems for the study of liver fibrosis in visceral leishmaniasis are defined, we may gain information of comparable significance to that which has already been gained for the cellular inflammatory immunopathology of this disease.

Helminthic Liver Diseases

Schistosomes are blood flukes that are well adapted to long survival as male and female adults in the venous circulations of human hosts. Human disease involves host responses to the deposition of schistosome eggs in tissues.

Fascioliasis is caused by flukes that primarily infect sheep and other herbivores. When humans are infected, a biphasic liver disease results from maturation of the parasite as it migrates through the liver parenchyma followed by an extended life span in the bile ducts.

Clonorchiasis and opisthorchiasis are biliary infections by trematode flukes that are well adapted to humans. Asymptomatic or minimally symptomatic for many years in most infected persons, these infections are of major concern because of their potential for the development of cholangiocarcinoma.

Table 49.1. Imaging Characteristics in Helminthic Liver Diseases

Infection	Imaging methods	Findings	Reversibility
Schistosomiasis	Ultrasonography	Portal fibrosis	Years to permanent
Fascioliasis, acute	Computed tomography	Serpiginous linear subcapsular abscess tracts	Months
Fascioliasis, chronic	Ultrasonography, computed tomography, cholangiography	Dilated ducts, visible adult worms	Months to years
Clonorchiasis and opisthorchiasis	Ultrasonography, computed tomography, cholangiography	Dilated irregular ducts, stones, associated cholangiocarcinoma	Years to permanent
Cystic and alveolar echinococcosis	Ultrasonography, computed tomography, magnetic resonance imaging	Cysts with variable wall calcification, complex internal structures, and daughter cysts	Years to permanent
Ascariasis	Ultrasonography, computed tomography, cholangiography	Dilated ducts obstructed by worms	Months

Echinococcosis is a potentially life-threatening cystic liver disease caused by the infection of humans as accidental intermediate hosts of three species of canine cestode tapeworms.

A relatively uncommon complication of intestinal infection with the nematode roundworm *Ascaris lumbricoides* is biliary ascariasis, which manifests as biliary colic, cholangitis, or pancreatitis induced by the migration of one or more of these large (up to 20 cm) adult worms into the ductal system (32,33,34). Biliary ascariasis usually occurs in children or in adults with an abnormal, open ampullary orifice produced by preexisting biliary tract disease or after surgical or endoscopic sphincterotomy (35). *Ascaris* worms in the ductal system are readily visualized on ultrasonography or computed tomography. If the worms do not spontaneously clear from the duct after antihelminthic treatment with mebendazole or an alternative agent, they may be removed endoscopically (32,35). Chronic biliary ascariasis has been implicated in the development of Oriental cholangiohepatitis, as discussed later for clonorchiasis and opisthorchiasis.

Abdominal imaging methods may show characteristic findings in helminthic liver diseases, as summarized in Table 49.1. The laboratory diagnosis of helminthic infections, as shown in Table 49.2, relies primarily on demonstrating eggs in the stool when the parasite's life cycle involves egg excretion by the human host. Serologic examinations, especially enzyme-linked immunosorbent assay (ELISA) methods, have become established for most infections as diagnostic adjuncts; serologic diagnosis is especially helpful when positive in the acute stage of fascioliasis and in echinococcosis, situations in which fecal egg excretion does not take place. Eosinophilia is such a regular accompaniment of most helminthic infections that its occurrence should prompt their diagnostic consideration, and its persistence after presumed parasitologic cure may signal treatment failure.

Table 49.2. Diagnosis of Helminthic Liver Diseases

Infection	Stool examination for eggs	Serology
Schistosomiasis	Method of choice in active infection, may be supplemented with rectal mucosal biopsy	ELISA available
Fascioliasis, acute	Negative	ELISA highly sensitive and specific, serial testing useful to monitor response to therapy
Fascioliasis, chronic	Often positive but egg production may be intermittent	ELISA method useful in addition to stool examination
Clonorchiasis, opisthorchiasis	Method of choice in active infection, stool PCR may be useful in mass screening	ELISA available; limited utility
Echinococcosis	Negative	IHA or ELISA positive in 90% of cases, serial testing useful to monitor response to therapy
Ascariasis	Method of choice	Not applicable

ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; IHA, indirect hemagglutination assay.

Schistosomiasis

Schistosomes are trematode flukes, which infect more than 200 million persons worldwide. Schistosomiasis has attracted more study and effort than all the other parasitic liver diseases combined. Few other diseases of any cause have posed the intensity and breadth of challenges in molecular biology, immunology, economic development, pharmacology, and surgical therapy that have been overcome and that remain for this disease (36).

Disease mechanisms

Schistosomes begin their life cycles with the passage of eggs by adult females that live, paired with male worms, in the mesenteric or vesical venous beds. Viable eggs erode through the intestinal or bladder mucosa, are passed in feces or urine, hatch in water, and infect an intermediate snail host. Snails shed free-swimming ceracariae, the infectious stage for humans, which have the ability to penetrate human skin and transform into immature worms. The worms mature over a period of approximately 6 weeks as they traverse the venous, pulmonary, and systemic circulations and localize in their species-specific target vessels to form male–female copulating adult worm pairs and initiate egg production that may continue for decades (37).

Five species of schistosomes, listed in Table 49.3, develop to maturity in humans. The great majority of schistosomal liver disease is caused by infections with *Schistosoma mansoni* in Africa and South America and *Schistosoma japonicum* in Asia. Although portal-tract egg deposition, granuloma formation, and fibrosis have been reported in persons infected with *Schistosoma hematobium* (38), these findings are minor compared with urinary tract egg deposition and disease. *Schistosoma mekongi* and *Schistosoma intercalatum* have limited geographic distributions in Asia and Africa, respectively.

Table 49.3. Human Schistosomes

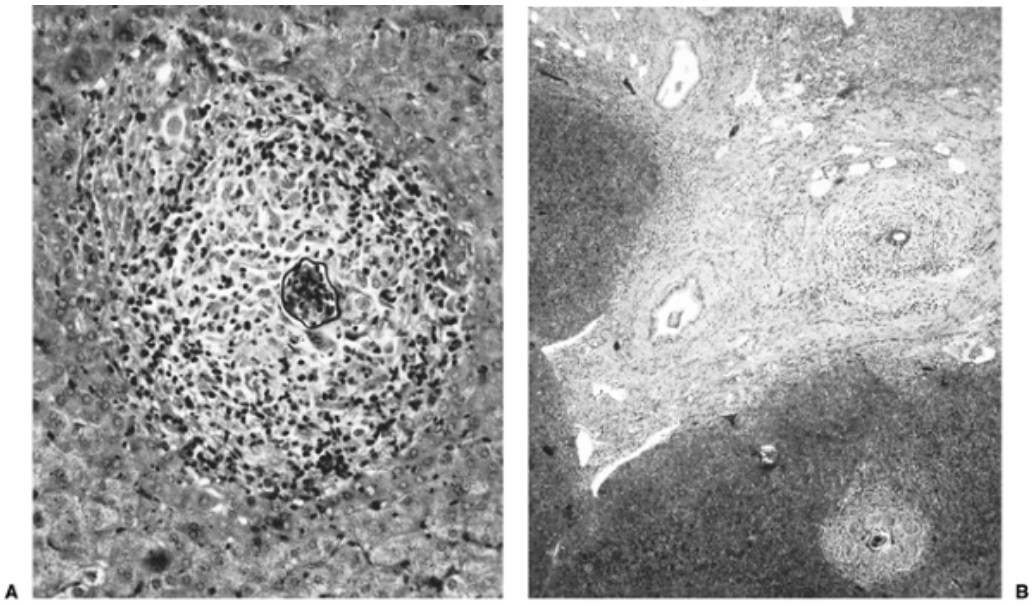
Species	Geographic range	Preferred vascular bed	Main target organs
Schistosoma mansoni	Middle East, Africa, Central and South America	Mesenteric	Liver, colon
Schistosoma japonicum	Far East	Mesenteric	Liver, small intestine, colon
Schistosoma hematobium	Middle East, Africa	Vesical	Bladder, ureters
Schistosoma mekongi	Southeast Asia	Mesenteric	Liver, small intestine, colon
Schistosoma	Central Africa	Mesenteric	Colon, less

intercalatum			severe liver and small intestinal disease

The preferential homing and predilection of maturing schistosomes of all species other than *S. hematobium* to concentrate in the mesenteric venous system, and of the latter species to concentrate in the vesical plexus, is central to the pattern of subsequent injury that their infections produce. Immature worms make several passes through the circulation before remaining at their preferred location (39). The mechanism that signals this remarkable preference for specific vascular beds is unknown. One potential localizing signal was suggested by the finding that human portal serum, but not peripheral blood, contains material of molecular weight greater than 1,000 that stimulates cell proliferation in immature *S. mansoni* worms (40).

Mature worm pairs in the mesenteric veins continuously produce large numbers of viable eggs that are carried to the intestine or the liver. Eggs deposited in the vessels of the intestinal mucosa may remain trapped within inflammatory granulomas, or erode into the

lumen and be excreted. Liver disease in schistosomiasis results from the entrapment of eggs that lodge in portal venules (41). Eggs in the liver remain viable for approximately 3 weeks and secrete products that elicit a characteristic initial response, the schistosome egg granuloma, as shown in Figure 49.2A. In some persons with heavy infections, the end result of hepatic schistosomiasis is severe portal fibrosis, as shown in Figure 49.2B. Advanced schistosomal hepatic fibrosis gives a gross appearance of greatly enlarged fibrotic portal tracts, described by Symmers (42) in 1904 as resembling clay pipestems thrust through the liver, and now termed Symmers' pipestem fibrosis.



▪ **Figure 49.2 A:** A *Schistosoma mansoni* egg granuloma in the liver. The schistosome egg has lodged in a portal venule, with formation of an epithelioid granuloma (hematoxylin-eosin, ×250). (AFIP Negative 79-16805.) **B:** Hepatic schistosomiasis. The large area of scarring would correspond grossly to pipestem portal fibrosis. A *Schistosoma mansoni* egg granuloma is at the *lower right* margin of the field (hematoxylin-eosin, ×60). (AFIP Negative 79-16808.)

Schistosomiasis has become a valuable model disease, in both clinical and experimental animal studies, for advancing our understanding of the key processes of hepatic inflammation and fibrosis (43,44,45). Important control points and mechanisms of immune regulation and collagen gene expression are more clearly defined in schistosomiasis than in other chronic liver diseases. The antigenic products secreted by living schistosome eggs first elicit a predominant Th 1–type cellular response, marked by an influx of mononuclear cells and formation of highly cellular egg granulomas, with initiation of increased collagen formation. Over time, from several weeks to months depending on the specific experimental model or human infection, a modulation of the initial cellular reaction takes place as egg deposition continues, with a diminution of the intensity of inflammation and a shift to a predominantly Th 2–type cellular response with prominent eosinophilic infiltration of granulomas and continuing deposition of fibrous tissue. Of the mediators associated with the Th 2 response, IL-13 appears to have strong potential as a pivotal mediator of fibrogenesis, based on studies in *S. mansoni* infected gene knockout mice that fail to express either IL-13 or its receptor complex (45). In humans, there appears to be a genetic component of susceptibility of infected persons to severe fibrotic disease. One report suggested an association between schistosomal hepatosplenomegaly and human leukocyte antigen (HLA) alleles A1 and B5 (46). In another study, a codominant gene with an allele frequency of 0.16 in a heavily infected community in Sudan was associated with severe schistosomal hepatic fibrosis. The gene, located on chromosome 6, was closely linked to the interferon-γ receptor gene (47). In general, however, the most important single determinant of the severity of disease in hepatic schistosomiasis appears to be the intensity of egg deposition in the liver over time (37).

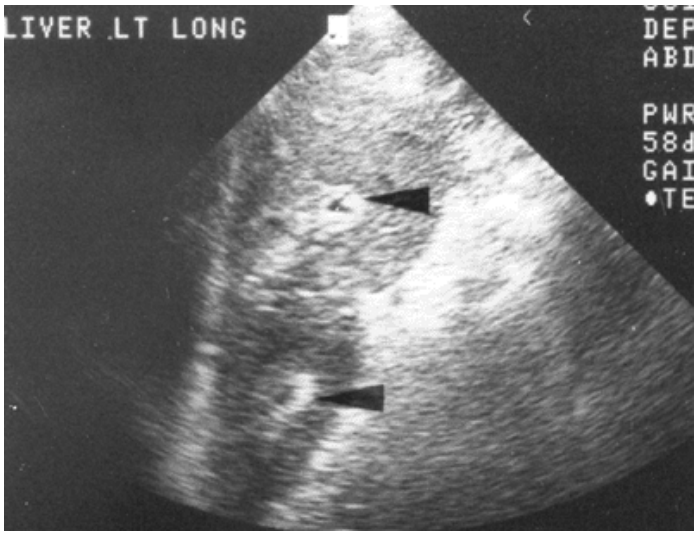
Hepatic fibrosis

Synthesis, deposition, remodeling, and turnover of collagen types I and III and basement membrane–associated collagen components, as well as that of fibronectin

and accompanying matrix substances such as glycosaminoglycans, are greatly increased in hepatic schistosomiasis, as are the plasma levels of multiple markers of collagen deposition. Experimental schistosomiasis has been an especially useful model for the study of the specific inflammatory cytokines proposed to have an influence on fibrogenesis (45).

In schistosomiasis, cells in granulomas and in adjacent fibrotic portal tracts show the appearance of stellate cells known to produce collagen and other connective tissue components in other chronic liver diseases. The potential interactions between granuloma macrophages, cytokines, and stellate cells appear to parallel those described in other experimental model systems (48). As disease progresses from initial egg deposition to advanced fibrosis, portal tracts become less prominently involved with inflammatory cells, and prominent cellular infiltrates diminish and disappear. Portal tracts in persons with Symmers fibrosis are markedly expanded with broad, dense-appearing bands of relatively acellular, mature fibrous tissue, as shown in Figure 49.2B.

Because normal liver architecture is preserved in hepatic schistosomiasis, reversal of portal-tract inflammation and fibrosis should allow resolution of the disease and restoration of normal function. Reversal of fibrosis has been well described after the cure of early *S. mansoni* and *S. japonicum* infections in mice (49,50). Murine schistosomiasis is one of the best-studied examples of the increased activity of two competing processes, collagen biosynthesis versus collagenolysis, in inflammatory fibrotic liver disease (51,52,53). In this model system, cure of infection with cessation of new egg deposition in the liver appears to allow collagenolysis to predominate over continued collagen synthesis, with resolution of fibrosis. It is unclear, however, to what extent the advanced dense portal collagen deposition associated with chronic human hepatic fibrosis might be subject to the same outcome. Two lines of evidence suggest that even dense pipestem fibrosis may be reversible, at least in part. First, rabbits infected with *S. japonicum* provide an animal model of dense portal collagen deposition that resembles human pipestem fibrosis morphologically and biochemically and shows slow reversibility of fibrosis over a 40-week period after cure of the infection (54). Second, serial ultrasonographic examination of persons with schistosome infection has become a standard method of assessing pipestem hepatic fibrosis in population-based treatment studies (55,56,57,58). The ultrasonographic appearance of pipestem fibrosis is shown in Figure 49.3. Ultrasonography in persons with acute nonfibrotic liver diseases may show a modest degree of portal-tract expansion that cannot be distinguished from early schistosomal fibrosis (59), and the imaging method is not reliable for assessing fibrosis in persons with schistosomiasis and coexisting conditions such as chronic viral hepatitis (60). Taking these precautions into account, multiple reports now clearly document the partial or complete resolution over several years of the ultrasonographic findings of pipestem fibrosis after parasitologic cure of *S. mansoni* or *S. japonicum* infection (61,62,63,64,65). In children and in adults treated after relatively short durations of infection, ultrasonographic resolution is more likely to be complete and accompanied by reversal of hepatomegaly and splenomegaly as assessed on physical examination.



▪ **Figure 49.3** Hepatic schistosomiasis. Ultrasonography shows echogenic deposits of pipestem portal fibrosis at *arrowheads*. (Courtesy of Colonel Michael P. Brazaitis, Department of Radiology, Walter Reed Army Medical Center.)

Clinical manifestations

The cercariae of all schistosomes, including those that die on skin penetration in humans, such as the avian schistosome species, may cause a hypersensitivity dermatitis, swimmer's itch. A potentially fatal acute illness, Katayama fever, is a serum sickness–like syndrome triggered by the onset of tissue egg deposition in heavy infections (36). The cardinal characteristic manifestations of advanced hepatic schistosomiasis are related to portal fibrosis and the development of presinusoidal portal hypertension, with passive congestion of the portal system, hepatomegaly, potentially marked splenomegaly, and the enlargement of collateral vessels such as esophageal and gastric varices. Patients classically present with a history of one multiple variceal bleeding episode, accompanied by prominent splenomegaly, no ascites, and normal or nearly normal indices of synthetic liver function and other biochemical laboratory values. It has become increasingly evident from careful longitudinal study of the populations of endemic areas, however, that the greatest health and economic impact of chronic schistosomiasis may

not be its dramatic end state with variceal bleeding, but retardation of growth and development, malnutrition, and generalized debility in heavily infected children and adults (66,67). Growth retardation in children with schistosomiasis is specifically associated with the infection rather than other potential causes and is only partially overcome after parasitologic cure (66).

The prominent splenomegaly of persons with hepatic schistosomiasis is caused both by infiltration with inflammatory cells and by passive congestion. The spleen is firm, normally nontender on palpation, and may be the site of sufficient sequestration to produce clinically important reductions in red blood cells, leukocytes, and platelets, as well as significant discomfort attributed to the bulk of the enlarged organ. Symptomatic splenomegaly may persist after the cure of infection, so that simple splenectomy is one of the most common surgical procedures in endemic areas. Segmental splenectomy, with removal of the bulk of an enlarged organ and preservation of a functional remnant of approximately normal size, is safe and

effective in experienced hands when hypersplenism in schistosomiasis requires surgical therapy (68). When variceal bleeding is an additional concern, portal variceal disconnection may be added (69), although as discussed in the subsequent text, optimal therapy to prevent recurrent variceal bleeding in schistosomiasis is far from clear. Massive splenomegaly may suggest the presence of follicular lymphoma of the spleen, the only malignant tumor clearly associated with hepatic schistosomiasis. Follicular lymphoma was reported to occur in 1% of *S. mansoni*–infected Brazilian patients who required splenectomy (70).

Bacterial infections associated with schistosomiasis include pyogenic liver abscesses, predominantly caused by *Staphylococcus aureus* (71) and chronic *Salmonella* bacteremia (72). Liver abscesses tend to occur in persons with early schistosome infections, perhaps coincident with the effects of initial egg deposition and the initial formation of highly cellular and vascular egg granulomas, as noted in the preceding text. Chronic *Salmonella* bacteremia appears related to the sequestration of living bacteria in the integument of adult worms and may be permanently cured only after elimination of the parasitic infection. HIV and schistosome infections now coexist in a growing number of persons as the HIV epidemic spreads through areas endemic for schistosomiasis. No clear-cut clinical interaction of these diseases, with their profound cellular immunologic disturbances, has been reported so far in persons with both infections (73).

Schistosomiasis and viral hepatitis

The accepted clinical findings of hepatic schistosomiasis—normal liver architecture and cellular function in the presence of portal fibrosis and portal hypertension—are present only in a minority of schistosome-infected persons who require hospitalization for liver disease. Because testing for the markers of hepatitis B and C infections has become widespread, it is evident that regions with a high prevalence of *S. mansoni* and *S. japonicum* infections also tend to have high endemicity for chronic viral hepatitis (74,75,76,77). In most populations studied, there is no higher occurrence of coinfection with schistosomiasis and hepatitis B or C than would be expected by their independent prevalence (78,79). An increased risk for acquiring both infections has been reported, however, in persons with schistosomiasis who required transfusions, and a major increase in the risk for hepatitis C infection has now become evident as the unintended consequence of mass treatment programs for schistosomiasis prior to 1980 that used injections with inadequately sterilized nondisposable syringes and needles (80). For example, in communities in Egypt with an overall prevalence of anti-hepatitis C virus (HCV) of 15% to 20%, up to 50% of persons in age groups with a history of such mass parenteral therapy are now anti-HCV positive (81). The intensive viral transmission attributed to these mass parenteral treatment programs, with formation of a large reservoir of chronic HCV infection, is thought to be responsible for the high current prevalence and transmission rates of hepatitis C in these areas (81).

In persons who develop both hepatic schistosomiasis and chronic viral hepatitis, severe illness is common. Most of the subset of persons living in schistosomiasis endemic areas who are hospitalized for variceal bleeding, management of ascites, or decompensated hepatocellular failure do, in fact, have both schistosomiasis and chronic viral hepatitis, frequently with cirrhosis (74,75,82,83). Whether comorbidity involves specific interactions of the pathologic mechanisms of both diseases or is simply a summation of their effects is unclear. Two comparisons of the extent of pathologic findings of chronic hepatitis C in liver biopsy specimens from persons with and without schistosomiasis suggested more severe histologic activity of chronic hepatitis in dually infected persons in one report (84), but not in the other (85). When acute hepatitis C infection occurred in health care providers with preexisting

chronic *S. mansoni* infection, they showed uniform inability to clear viremia and accelerated histologic progression of chronic hepatitis C compared with the course of hepatitis C infection in their colleagues without schistosome infection (86). Persons with coexisting *S. mansoni* infection and chronic hepatitis C appear less likely than others to respond to interferon therapy (87,88) and *S. mansoni*–infected persons respond less consistently to hepatitis B vaccination (89,90), although vaccination should be a high priority in schistosome-endemic areas to prevent as

much comorbidity as possible. Antiviral therapy for chronic hepatitis B or C should be considered for persons who have been cured of active schistosome infection and who would otherwise meet treatment criteria, bearing in mind that the predominant HCV genotype transmitted in Egypt is type 4 (88).

Diagnosis

Detection of schistosome eggs in the stool is the most useful diagnostic method for documenting active infection. Quantitative studies have shown generally strong relationships between the extent of fecal egg output, the host's worm burden, and the extent of pathology in both *S. mansoni* and *S. japonicum* infections (37,41). Stool examinations for eggs become negative after parasitologic cure of infection. Some persons with active untreated *S. japonicum* infections causing significant morbidity may also show few or no eggs on stool examination (66). Low-power examination of fresh rectal mucosal biopsies may show schistosome eggs that were not apparent in stool specimens. Serologic ELISA methods to detect the antibody to parasitic antigens show excellent sensitivity and are of value in population surveys, but may not be helpful in assessing the activity of infection in an individual patient (91). Of the standard abdominal imaging methods, ultrasonography, as discussed earlier, is by far the most practical for field application and appears to be of better diagnostic utility than computed tomography scan (92) or magnetic resonance imaging (93) for assessment of the extent of portal fibrosis.

Medical therapy

Praziquantel is an effective drug against all human schistosomes, producing parasitologic cures in approximately 90% of persons (37). It is orally administered, preferably in three doses of 20 mg/kg body weight given over 8 hours for a total of 60 mg/kg. Single-dose therapy of 40 and 50 mg/kg has been used in some community mass treatment programs for *S. mansoni* and *S. japonicum* infections, respectively (37). Praziquantel is approved by the U.S. Food and Drug Administration (FDA) for use in schistosomiasis. Gastrointestinal irritation is the major side effect of this generally well-tolerated drug. Another drug effective for *S. mansoni* infection is oxamniquine, used in mass treatment programs in Africa and South America.

Mass treatment programs have become a central element in the efforts of many countries to combat schistosomiasis. For *S. mansoni* infection, a community-based strategy of mass chemotherapy followed by periodic surveillance with stool examinations and prompt treatment of any reinfections has reduced morbidity and promoted resolution of ultrasonographic evidence of portal fibrosis (61,63,64). Reducing new transmission by largely eliminating the contamination of water with shed parasite eggs underlies this approach. Although promising results have also been achieved in some *S. japonicum*–endemic areas (62,65), adults in other treated communities show persistence of hepatosplenomegaly and fibrosis even with decreased prevalence of infection (94). The existence of numerous animal reservoirs of *S. japonicum* in other communities, coupled with the inability of stool examinations to detect all *S. japonicum* reinfections, suggests that in these locations, frequent mass retreatment would be more effective than surveillance by

stool examination for reinfection (66,94). In addition, parasitologic cure followed by prompt reinfection might produce a rebound of the relatively severe inflammatory and fibrotic events that accompany acute infection rather than the persistence of a modulated, relatively less damaging long-term response (66). In such a situation, it may be especially important to maintain effective mass retreatment once a decision has been made to begin community-based therapy.

There are no clinical data to suggest that specific therapy promotes any greater degree of resolution of schistosomal liver fibrosis in humans beyond what would normally be expected after the cure of active infection, as discussed in the preceding text. However, administration of the immunomodulating Th 1 cytokine interferon-γ to schistosome-infected mice, or promotion of its release by the inhibition of IL-4, diminishes liver collagen deposition (95,96). Schistosomes, along with all other multicellular parasites, continue to defy efforts to produce an effective antiparasitic vaccine. However, a combined antigen–cytokine vaccination concept may help limit parasite-induced host injury: Sensitization of mice with *S. mansoni* eggs administered together with IL-12 appears to prime the animals to respond with markedly diminished liver fibrosis when they are subsequently challenged with a schistosome infection (97).

Surgical therapy

Four major factors contribute to portal hypertension, increased collateral blood flow, and variceal enlargement in schistosomiasis (98,99). They include portal fibrosis that produces presinusoidal obstruction of portal inflow; arterialization of abnormal vessels within the portal fibrous tissue that promotes increased hepatic arterial inflow; cellular infiltration of the spleen that adds to passive congestion to produce marked splenomegaly and increased splenic blood flow; and poorly understood functional disturbances that diminish splanchnic resistance and promote a hyperdynamic splanchnic circulation.

Nearly every form of medical and surgical therapy for bleeding varices has been advocated in schistosomiasis. Solid information to support evidence-based

treatment decisions remains as elusive in schistosomiasis as it is for other causes of variceal bleeding. The results at 4 to 10 years of an important randomized trial of proximal splenorenal shunt, distal splenorenal shunt, and splenectomy with esophagogastric devascularization in schistosomiasis have been reported (100) and critically reviewed (101). Bleeding recurred in one fourth of patients in each surgical treatment group, with slightly more variceal bleeding after splenectomy-devascularization. Occurrence of mortality and hepatic encephalopathy, both approximately 40%, was much greater after proximal shunting than after either distal shunting, with 15% mortality and 15% encephalopathy, or splenectomy-devascularization, with 7% mortality and no encephalopathy. Taking into account the study's reported limitations, the data support the current reliance on splenectomy-devascularization in many centers as the preferred surgical option for variceal bleeding refractory to endoscopic therapy. The procedure may involve either total or segmental splenectomy as discussed earlier. As endoscopic control of variceal bleeding with band ligation and sclerotherapy becomes more effectively practiced and widespread, surgical therapy for bleeding varices is likely to become increasingly limited to devascularization in persons who require splenectomy for other indications, as discussed in the preceding text.

The benefits and potential problems of endoscopic sclerotherapy of bleeding varices are similar for schistosomiasis and other liver diseases (102,103,104,105). Growing experience with endoscopic band ligation of varices in schistosomiasis has been encouraging (106,107). A randomized study of band ligation versus sclerotherapy in

40 Brazilian patients reported similar high efficacy and safety for both procedures, with better patient acceptance of band ligation, and a trend toward fewer sessions required for variceal eradication with band ligation (106).

Two controlled secondary prevention trials of propranolol in patients with schistosomiasis and variceal bleeding have shown decreased recurrence of bleeding (108,109). One of these reports from Sudan, which evaluated sustained-release propranalol at a single daily dose of 160 mg, showed a 40% reduction in mortality after 2 years (109). β-Blockade appears to be a useful adjunct for secondary prevention of variceal bleeding in schistosomiasis, in line with its value in other liver diseases, and to merit consideration for evaluation in primary prevention.

The general movement toward medical, endoscopic, and nonshunting surgical therapy for variceal bleeding in schistosomiasis shares common ground with the same trends and uncertainties that apply to other liver diseases. Key considerations in schistosomiasis include the frequent occurrence of marked splenomegaly that has attracted interest in evaluating the potential benefits of complete or partial splenectomy, as well as concern about encephalopathy after shunting. Shunted patients require lifelong vigilant prevention of reinfection to avoid iatrogenic pulmonary egg deposition and the development of schistosomal-induced cor pulmonale. Pulmonary hypertension is a known problem even in nonshunted persons with hepatic schistosomiasis, potentially related to incomplete hepatic clearance of vasoactive compounds and mediators (110).

Fascioliasis

Fasciola hepatica is a trematode bile duct fluke with a worldwide distribution in sheep and cattle (111). The leaf-shaped male and female adult worms reach a size of approximately 2 cm and may remain viable in the bile ducts for more than a decade. They produce eggs that pass in feces, hatch in water, and infect a snail as an intermediate host. Snails release a cercarial stage of the parasite that contaminates aquatic plants ingested by sheep, cattle, or humans. When ingested, transformed metacercariae penetrate the intestine, traverse the peritoneal cavity and liver capsule, and burrow through the liver parenchyma for 1 to 3 months while maturing, finally entering the bile ducts to become mature adults and complete the cycle. Heavy infection of sheep and cattle, called *liver rot*, is an important economic cause of livestock loss in areas where animals regularly consume aquatic vegetation. Humans with fascioliasis generally give a history of eating watercress or drinking potentially contaminated water. Human fascioliasis is prevalent in developing countries with humid climates and largely agrarian populations and is less frequently seen in Europe and North America. In the Nile Delta of Egypt, there is a positive association between *Fasciola* and *S. mansoni* infections (112).

Acute fascioliasis is a febrile illness, typically of up to 3 months duration, presenting with right upper quadrant discomfort and hepatomegaly. As immature flukes continue their course through the liver parenchyma, they leave behind a track of coagulation necrosis infiltrated by an intense eosinophilic inflammatory response. In sheep with experimental fascioliasis, the ability of the parasite to keep moving ahead of the host inflammatory response appears to allow it to literally outrun what would otherwise be an effective host defense (113). The resulting tracks show a characteristic appearance of yellow–white serpiginous subcapsular cords at laparoscopy (114). On computed tomography, the tracks appear as tortuous linear arrays of small, 1- to 3-cm abscess-like lesions (115).

In acute fascioliasis, fever, leukocytosis, and right upper quadrant pain are each present in approximately two thirds of patients. In 20 patients reported from

Spain, 19 had eosinophilia in the 15% to 65% range (111). Atypical manifestations of acute fascioliasis may result when penetration of the ductal system causes hemobilia or discrete abscess formation (116). Immature flukes that fail to migrate into the liver can produce ectopic masses or abscesses in many locations, most commonly appearing as subcutaneous nodules with an eosinophilic infiltrate surrounding the degenerating parasite tissues. In addition, a syndrome of eosinophilic pleuritis and pericarditis without direct parasitic involvement of these structures may accompany acute fascioliasis (111).

After bile duct penetration by mature flukes, egg production initiates chronic fascioliasis. Host responses to adult worms are limited to local inflammation, ductal epithelial proliferation, and fibrous thickening of the duct wall. *Fasciola* produces proline, a key precursor of collagen, as a major nitrogen excretion product. Animal experiments suggest that high local concentrations of proline in fascioliasis may promote ductal hyperplasia and fibrosis (117). Large numbers of adult flukes in the ductal system may precipitate episodes of acute biliary obstruction and cholangitis (111). In addition to visualization of the adult flukes, dilated ducts may be seen by ultrasonography, computed tomography, or cholangiography.

Fever and right upper quadrant abdominal pain in acute fascioliasis, or biliary symptoms in chronic infection, coupled with a consistent dietary history, suggest the possibility of fascioliasis. Eosinophilia and consistent imaging findings as discussed in the preceding text strongly support the diagnosis. Serologic testing for antibody to the parasite was refined by development of an ELISA method using a purified preparation of a *Fasciola*-specific protease, cathepsin L1, as antigen (118). The assay detected 20 of 26 persons with fascioliasis, with no false positive tests in persons with other helminth infections. An alternative approach using a crude *Fasciola* worm antigen preparation achieved 97% sensitivity; high specificity of this method required identification of immunoglobulin G (IgG)4 subclass antibody (119). Antibody levels slowly decrease after successful treatment (120). Stool examination for eggs is useful only in chronic disease and may be negative when egg output is intermittent.

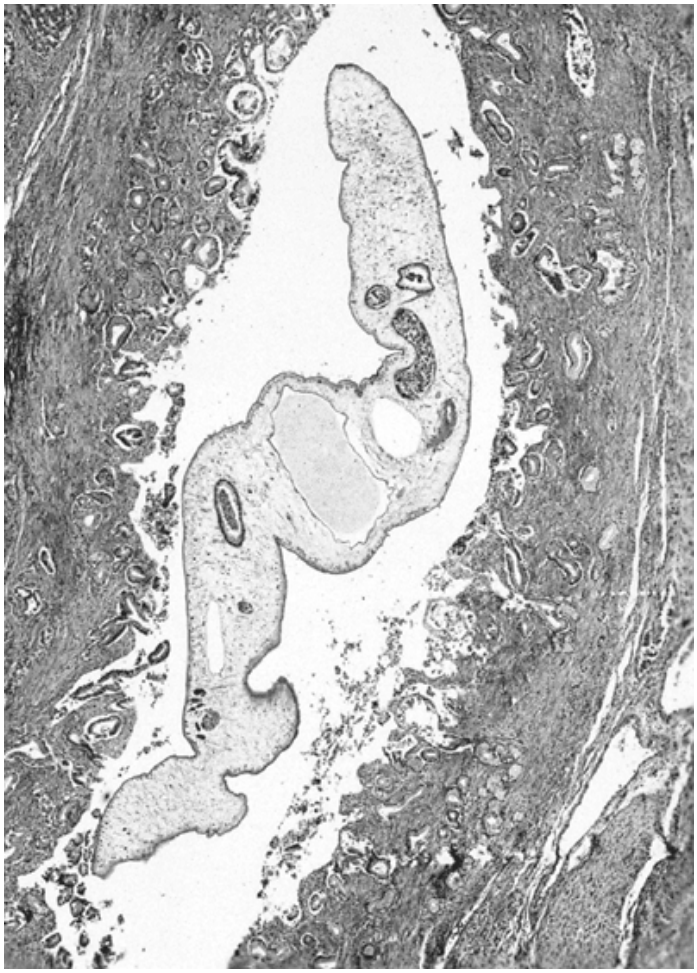
Bithionol has been the most widely used antihelminthic compound for fascioliasis. It is given in courses of 10 to 30 days for acute and chronic infections (111). Gastrointestinal irritation is common; rash, leukopenia, and hepatotoxicity are less frequent; and retreatment may be attempted for nonresponders to an initial course of therapy. Triclabendazole, a benzamidazole compound widely used in veterinary practice, appears to be more effective and less toxic than bithionol in humans with fascioliasis (121). Limited quantities of bithionol are available in the United States from the Centers for Disease Control and Prevention parasitic drug service as an investigational drug. Triclabendazole, the preferred agent, may now be imported for investigational human use in the United States with FDA approval of an expedited individual use request. A 3- week course of oral metronidazole therapy was recently reported to be effective in Iranian patients who had not responded to triclabendazole (122).

Clonorchiasis and Opisthorchiasis

Clonorchis sinensis, *Opisthorchis viverrini*, and *Opisthorchis felineus* are bile duct flukes acquired by humans who eat raw fish containing the parasite's infective metacercaria stage. Human hosts excrete eggs that hatch in water and pass through snail and fish stages to infect new humans and animals. With a size of approximately 1 cm and a ventral sucker that permits attachment to the intrahepatic bile duct epithelium, male and female adult flukes have life spans of 10 years or more. *C. sinensis* infects persons in China and elsewhere in east Asia; approximately one fourth of Chinese immigrants to New York City have active infection (123). *O.*

viverrini has a more limited range in Thailand, Laos, and Cambodia but shows very high prevalence in northeast Thailand, where one third of the population is infected (124). *O. felineus* infects cats and humans in limited areas of Russia and eastern Europe.

Most infected persons have relatively light parasite burdens of 100 or fewer worms. For *O. viverrini* infection, there is a strong quantitative relationship between the number of eggs excreted per gram of stool and a host's burden of adult worms, as determined at autopsy (125) or by retrieval of worms in stool after parasitologic cure of infection (126). A new polymerase chain reaction (PCR)-based fecal test as an alternative for microscopic stool examination may facilitate the screening of large populations (127). Study of the intensity of infection in population-based samples has provided strong evidence to support the linkage of bile duct fluke infection with chronic biliary tract abnormalities and with the ultimate development of cholangiocarcinoma, a leading cause of cancer deaths in endemic areas. In surveys using ultrasonography, the intensity of infection is strongly correlated with the occurrence of gallbladder enlargement and wall irregularity, biliary sludge, and enhanced portal-tract echogenicity (128). For cholangiocarcinoma in northeastern Thailand, a locality-adjusted odds ratio of 14.1 was found for male residents with the highest intensity of *O. viverrini* infection; 4% of the surveyed male population with more than 6,000 eggs/g feces reportedly had the malignancy (129).



▪ **Figure 49.4** Clonorchiasis. A dilated intrahepatic bile duct contains an adult worm cut in cross section. The ductal epithelium shows adenomatous

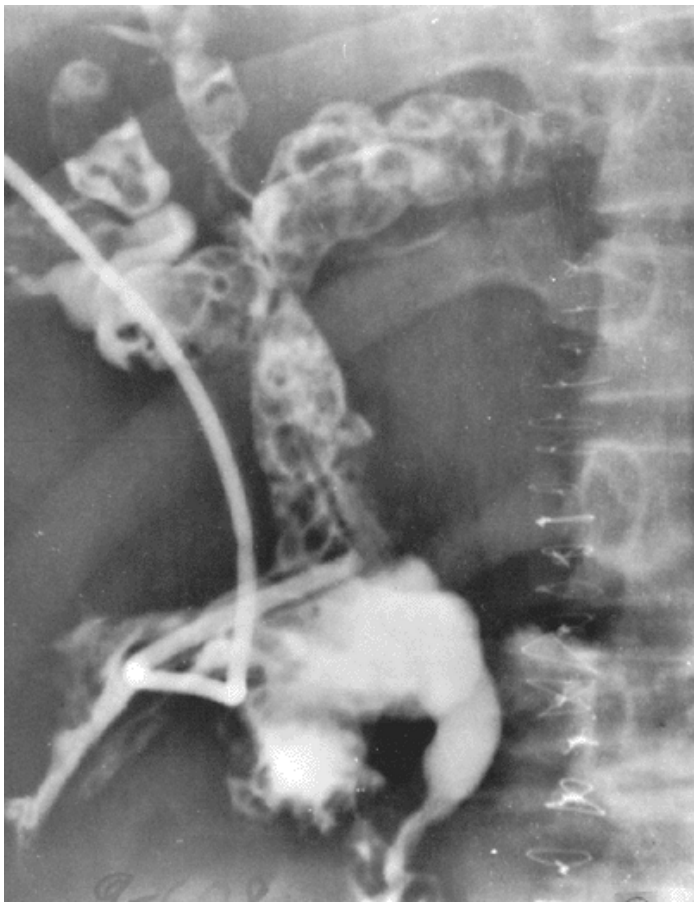
hyperplasia (hematoxylin-eosin, ×40). (AFIP Negative 72-11587.)

The bile ducts that harbor adult *Clonorchis* or *Opisthorchis* worms can show dilatation, irregular thickening, and adenomatous epithelial hyperplasia, as shown in Figure 49.4. Some of these changes may be reversible, especially after treatment of light or early infection. Ten months after eliminating *O. viverrini* infection with praziquantel treatment, repeated ultrasonographic study of 72 persons showed resolution of gallbladder enlargement, improved gallbladder contractility, and decreases in visible sludge and portal-tract echogenicity (130). However, repeat endoscopic cholangiography at an average interval of 32 months in persons treated for *C. sinensis* infection showed some improvement in the appearance of the intrahepatic ducts and loss of the filling defects caused by the presence of adult worms but no changes in measured duct enlargement or the presence of duct wall irregularities (131). Adenomatous hyperplasia of the papilla in chronic clonorchiasis can produce radiographic duct abnormalities indistinguishable from those of cholangiocarcinoma (132).

The development of cholangiocarcinoma may reflect the interaction of multiple processes. For example, *O. viverrini*–infected persons with ultrasonographic biliary abnormalities were shown to have increased activity of cytochrome P-450 2A6, an enzyme that promotes activation of carcinogenic nitrosamines (133). Similarly, administration of dimethylnitrosamine to *C. sinensis*–infected hamsters resulted in development of cholangiocarcinomas that did not occur in uninfected animals or in infected animals not exposed to the carcinogenic compound (134). Although the morbidity associated with biliary fluke infection before the development of cholangiocarcinoma appears to be slight in most persons, the outlook is very poor in those who present with the tumor, similar to that for the same disease in nonendemic areas (135). With currently available information, it seems appropriate to continue efforts to persuade residents of areas with high prevalence of infection and cholangiocarcinoma to modify their dietary habits, and to eliminate existing infection with praziquantel, an easily administered and effective curative drug (124). Screening and parasitologic cure of immigrants to Western countries, who are at high-risk for infection, is also warranted.

Oriental cholangiohepatitis is a chronic illness marked by episodes of cholangitis with formation of multiple pigment stones, irregular bile duct dilatation with disproportionate severity in the extrahepatic ductal system, and formation of multiple strictures (136,137). Its geographic range corresponds roughly to that of the bile duct flukes and *Ascaris* infection. Figure 49.5 shows a cholangiogram from a patient with

this condition and associated clonorchiasis. In many patients with severe distortion of the ductal system, recurrent episodes of cholangitis appear to be self-perpetuating in the absence of active parasitic infection. Helminth infection should be sought and eliminated in persons with the disease. In addition, recurrences due to obstructing stones may be more easily managed in patients who have had placement of a Roux-en-Y jejunal conduit for biliary access (138).



▪ **Figure 49.5** Oriental cholangiohepatitis associated with clonorchiasis. The cholangiogram shows a dilated, distorted intrahepatic ductal system with contrast material outlining numerous filling defects that represent adult *Clonorchis sinensis* flukes. (AFIP Negative 96-22949.)

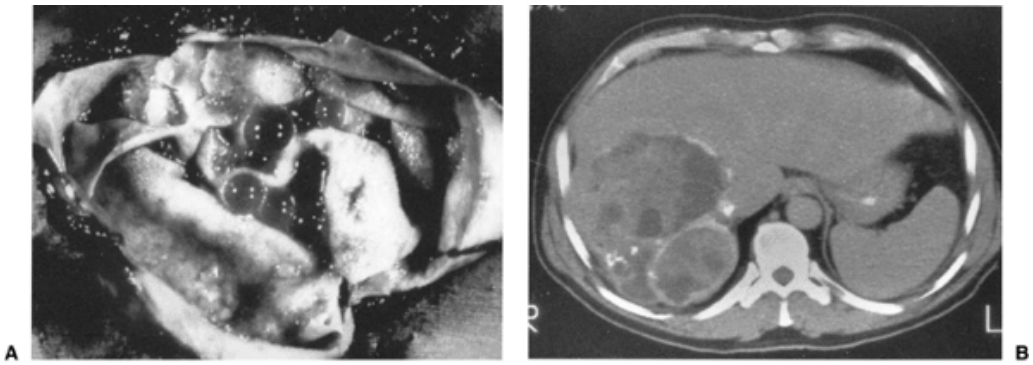
Echinococcosis

Echinococcosis, or hydatid disease, develops in humans when they become accidental hosts for a cystic intermediate stage of one of three canine tapeworms, *Echinococcus granulosus*, *Echinococcus multilocularis*, or *Echinococcus vogeli*. Humans become infected by eating food contaminated with eggs excreted by domestic or wild dogs or other canines such as foxes, coyotes, and wolves (139,140). The parasite normally multiplies as a larval scolex stage within cysts in the solid organs of herbivores or rodents that have consumed excreted eggs. Consumption of the cyst-containing viscera of these animals by new canines completes the cycle.

Hydatid disease most often affects humans in contact with sheep-herding dogs infected with *E. granulosus*. The resulting cystic hydatid disease has a worldwide distribution. *E. multilocularis* infection, concentrated mainly in the arctic and subarctic regions of the Northern Hemisphere, causes human alveolar hydatid disease. *E. vogeli*, the cause of human polycystic hydatid disease, has a very limited range in Central and South America (141).

Hydatid liver cysts caused by *E. granulosus*, as shown in Figure 49.6, are most often asymptomatic. They are fluid-filled structures delimited by a parasite-derived

membrane, as shown in Figure 49.7A, which contains germinal epithelium that buds viable scoleces, shown in Figure 49.7B. Imaging by ultrasonography, computed tomography (as shown in Figure 49.6), or magnetic resonance may demonstrate the formation of daughter cysts, cyst wall calcification, and compression and fibrous reaction of the surrounding liver parenchyma, and in complicated disease, communication of the cyst with the biliary system or external leakage of cyst material. The cysts formed in *E. multilocularis* infection are less well delimited. They tend to invade the liver parenchyma and seed adjacent organs and structures with scoleces and daughter cysts (142). The polycystic hydatid disease of *E. vogeli* infection shows well-delimited multiple cysts (141).



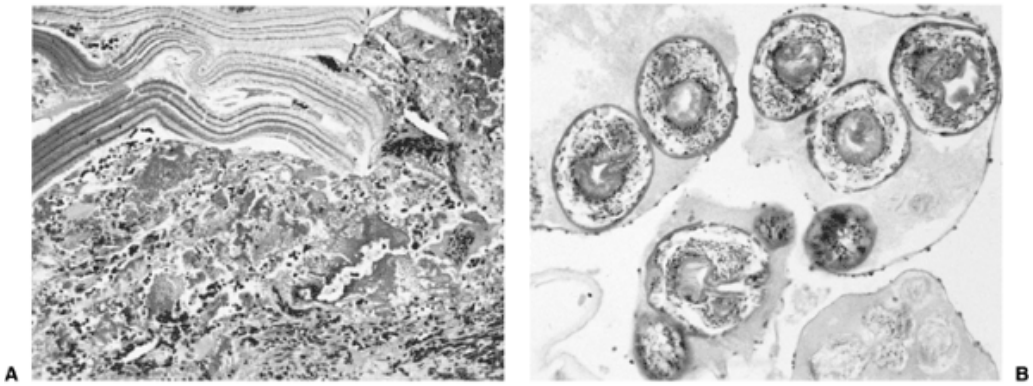
▪ **Figure 49.6 A:** Hepatic hydatid cyst of *Echinococcus granulosus*, opened to show folds of the pale thickened germinal membrane associated with translucent budding daughter cysts. (AFIP Negative 95-82642.) **B:** Hepatic hydatid cyst of *Echinococcus granulosus*. A computed tomographic image shows a large cyst in the posterior right lobe of the liver with a complex internal structure similar to the cyst shown in (A) and areas of wall calcification. (AFIP Negative 92-84646.)

Cystic hydatid disease now most often presents as a hepatic mass with a typical appearance on abdominal imaging, coupled with confirmatory serologic testing by indirect hemagglutination or ELISA, which is positive in approximately 90% of hydatid cysts (140). As discussed in the preceding text, no fecal eggs are present in human hosts. Eosinophilia is usually present. Biliary or peritoneal extension or pulmonary cystic disease is usually easily recognized; however, ectopic cysts in the kidney, spleen, brain, orbit, heart, and bone may produce unusual findings. Uncommon presentations of hydatid disease include segmental portal hypertension due to splenic vein compression by a cyst in the splenic hilum, with adjacent perihilar varix formation (143), and rupture of a hepatic cyst causing inferior vena cava thrombosis (144).

Until recently, most hydatid cysts came to clinical attention because of symptomatic enlargement, so that their management by surgical excision was a straightforward decision. Palliative resections for the spreading, poorly contained cysts of *E. multilocularis*–induced alveolar hydatid disease were often inadequate to deal with this frequently lethal disease.

Now that many hydatid cysts are detected as incidental findings during abdominal imaging and newer forms of medical and surgical therapy for hydatid disease are being reported, therapeutic options are at once more promising and more

challenging.



▪ **Figure 49.7 A:** Hepatic hydatid cyst of *Echinococcus granulosus*. Laminated membrane, top, and debris within the cyst (hematoxylin-eosin, ×160). (AFIP Negative 82-12615.) **B:** Hepatic hydatid cyst of *E. granulosus*, showing viable scoleces cut in cross section (hematoxylin-eosin, ×250). (AFIP Negative 82-12530.)

After initial evaluation of the benzimidazole antihelminthic compound mebendazole, a related compound, albendazole, has become the current standard for medical therapy of hydatid disease (145,146,147,148). Albendazole has strong scolicidal activity for *E. granulosus* and *E. multilocularis* and superior absorption, bioavailability, and distribution into hydatid cysts compared with mebendazole (145). Albendazole was reported to be an effective preoperative adjunct for disrupting the viability of *E. granulosus* and *E. multilocularis* cysts and improving resectability in the latter disease (142,146,147). Albendazole was studied as primary therapy in 59 persons with hepatic *E. granulosus* cysts of diameter 10 cm or less: After 3 to 7 years, cysts had fully resolved in 24 patients, decreased in size in another 24, not changed in 9, and recurred after initial resolution in 2 (148). In another series of 19 patients treated with a combination of albendazole and praziquantel for 2 to 6 months, the combination was reported to produce more rapid and complete resolution than that seen in 22 patients treated earlier by the authors using albendazole alone (149); however, the results of combination therapy in this report were similar to those reported by others for albendazole alone. In an extensive series of 929 cysts in 448 persons treated with mebendazole or albendazole with up to 14 years follow-up, approximately 1 in 4 cysts recurred after initial resolution or regression (150). Recurrence was greatest within 2 years of resolution. Albendazole, approved for use in the United States, is generally administered two to three times daily with food at doses ranging from 10 to 50 mg/kg per day, for 12 to 24 weeks or longer, with or without intervening rest periods (145,146,147,148). Toxicity includes variable alopecia and, in many patients, minor elevations in aminotransferase levels as well as transient pain perceived at the location of a cyst on initiation of treatment. Elevation of aminotransferase levels more than four times normal or leukopenia requires discontinuing albendazole.

Traditional surgical therapy for hepatic *E. granulosus* cysts at laparotomy includes isolation by packing; careful aspiration of cyst fluid to avoid spillage of viable scoleces or anaphylaxis; injection of the cyst with hypertonic saline, alcohol, or dilute silver nitrate to kill the scolices if the aspirated fluid is crystal clear; and

resection of either the cyst alone or both the cyst and its pericystic rim of compressed liver tissue (151,152). Aspiration of turbid cyst fluid suggests a biliary communication so that injection with potential sclerosants is avoided. The use of percutaneous drainage, described in the subsequent text, for an increasing proportion of patients with relatively simple cysts, has left the remaining patients who now come to open surgery as a population with relatively greater aggregate complexity and technical challenge than those of earlier surgical series.

Laparoscopic evacuation (153) and ultrasound-guided percutaneous drainage (154,155,156,157,158) are reported to be safe and effective for treating uncomplicated cystic hydatid disease. Percutaneous drainage has become the first-line management for cystic hydatid disease in many centers. The most extensive current experience is with an ultrasound-guided four-step process of puncture; aspiration; injection with hypertonic saline, silver nitrate, or other scolicidal solution; and re-aspiration (PAIR) method, with one or more days of subsequent percutaneous catheter drainage for

large cysts. Patients normally receive a several day preprocedure course of oral albendazole, and are given antihistamine and steroid coverage for the procedure to minimize anaphylaxis in the event of cyst leakage at puncture, followed by continued albendazole for 2 months after the procedure. Advantages of the PAIR technique include minimal disability and early return to full activity, with a 1- to 2-day length of hospital stay compared to a typical 2-week stay after open cyst evacuation. In contrast to primary medical therapy without aspiration and drainage, the PAIR technique has the advantages of a much shorter duration of 2 months of adjunctive albendazole therapy postprocedure, compared with up to 6 months or more for albendazole as primary treatment, as well as a minimal recurrence rate compared with recurrence of 25% of cysts treated medically alone (150). PAIR and laparoscopic cyst evacuation in experienced centers have both shown low morbidity, with anaphylaxis being the most significant problem in the 1% to 2% range, which is generally easily managed in pretreated patients, and the potential for bleeding or duct injury common to all liver punctures.

Patients with alveolar hydatid disease due to *E. multilocularis* have an infection whose biologic behavior resembles that of a malignant tumor as its complex cystic structures invade the liver parenchyma and spread by direct extension to adjacent sites. Albendazole therapy is administered in an effort to stabilize unresectable disease, or as an adjunct to liver resection, which may cure early localized disease. Alveolar hydatid disease may require multiple surgeries in an attempt to deal with severe complications such as hepatic vein compression and thrombosis, and secondary sclerosing cholangitis. Similar major problems occur much less frequently in cystic hydatid disease. Liver transplantation for patients with end-stage alveolar or cystic hydatid disease often presents a technical challenge related to extensive prior surgery, but transplantation may produce a good long-term outcome in patients whose disease has progressed beyond the point of cure or control by other methods (159,160).

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Chapter 50
Bacterial and Systemic Infections

Stuart C. Gordon

Key Concepts

- Involvement of the liver with various bacterial, fungal or rickettsial infections may be a primary disorder or part of a multisystemic disorder. These infections often mimic other conditions, therefore a high degree of clinical suspicion is often needed to establish the diagnosis of nonviral hepatic infection.
- Cholestasis and hyperbilirubinemia can occur after gram-positive or gram-negative bacteremia, even in the absence of fever or positive blood cultures, and is often an overlooked entity. Endotoxin-mediated cytokine release is the likely cause of septic jaundice.
- The possibility of a nonviral hepatic infectious disorder should be suspected when a patient with a fever has a cholestatic biochemical profile (predominant alkaline phosphatase elevation out of proportion to the aminotransferase derangement). Occupational and travel history may provide diagnostic clues, and results of specific serologic tests or biopsy of the liver may lead to the institution of appropriate therapy.
- Although in the past many nonviral hepatic infectious diseases were diagnosed retrospectively with serologic titers or microbiologic cultures, currently polymerase chain reaction (PCR) (candidiasis, tuberculosis, tularemia, leptospirosis, Q fever) is increasingly used to facilitate early diagnoses.

A variety of bacterial, fungal, and rickettsial infections affect the liver, either as the result of direct hepatocellular or biliary invasion or through the production of toxins. In addition, systemic infectious processes frequently cause jaundice or nonspecific liver biochemical abnormalities through mechanisms that are less defined. The jaundice of bacterial pneumonia has been long recognized (1,2), and both jaundice and aminotransferase abnormalities have been associated with other systemic infections, including appendicitis (3), bacteremia in infants (4,5), and other extrahepatic infections (6,7). Most descriptions of liver dysfunction during systemic infection were reported several decades ago, whereas recent research has focused on the mechanisms of the jaundice of sepsis. Reports of the hepatobiliary manifestations of specific infectious agents come from around the world; newly recognized manifestations, diagnostic modalities, and treatment regimens shed new light on the clinical relevance of these conditions. Anecdotal case reports emphasize the need for clinicians to consider the possibility of such infectious agents and entities when encountering unusual cases of hepatitis or cholestasis.

Bacterial Infection and Jaundice

The observation that extrahepatic bacterial infection can cause jaundice has been attributed to Garvin (8). Osler (9) in 1892 found that in patients with pneumonia, jaundice might occur. The syndrome of septic jaundice is highly variable and may range from nonspecific

biochemical cholestasis to deep jaundice. Sepsis as a cause of jaundice in the hospitalized patient is often an overlooked entity (10). A disproportionate elevation of serum bilirubin in comparison with serum alkaline phosphatase and aminotransferase levels should suggest underlying sepsis, even in the absence of fever or leukocytosis (11). Accordingly, early medical or surgical intervention may reduce morbidity and mortality (11).

In more than one third of patients with sepsis, hyperbilirubinemia associated with bacteremia with or without an increase in serum alkaline phosphatase level may occur 1 to 9 days before the initial positive blood culture result is obtained. Although gram-negative bacteria, especially *Escherichia coli*, have been implicated as the predominant causative agent in most series, infection with nonhepatic gram-positive organisms, especially *Staphylococcus aureus* (12,13) also have been cited.

The mechanisms by which bacterial infection causes cholestasis and jaundice are not clear, but endotoxemia appears to be the likely cause. Lipopolysaccharide (LPS), or endotoxin, is contained within the outer membranes of gram-negative bacteria and is a potent inducer of cytokines. Endotoxemia may occur in the absence of documented sepsis (14), and increased levels of tumor necrosis factor α and other cytokines occur in alcoholic hepatitis and in jaundice associated with total parenteral nutrition. Accordingly, endotoxin-mediated cytokine release may be the basis for the jaundice of many disorders, including sepsis (15,16).

Early studies showed a reduction in bile flow and biliary excretion after administration of endotoxin to isolated, perfused rat liver. Pretreatment with dexamethasone, which blocks endotoxin-mediated release of the cytokines tumor necrosis factor α and interleukin-1, largely prevented this reduction in bile flow (11,17). The cholestasis of sepsis has long suggested an impaired hepatocyte transport of bile acids and organic anions. Studies have shown that canalicular bile acid and organic anion transport are markedly impaired in endotoxemia (18). Therefore, endotoxemia severely impairs the transport of organic anions at both the sinusoidal and canalicular membrane. Because of impaired hepatic organic anion transport, both bile acid–dependent and bile acid–independent

components of bile flow decrease with the administration of endotoxin. Li et al. (19) recently demonstrated that LPS administration decreases organic anion transport mRNA levels in mice, and that this decrease is mediated through toll-like receptor 4 (TLR4).

The jaundice of sepsis was historically described as occurring in pediatric patients, but its presence in adults is increasingly observed. Marked elevations in direct and total serum bilirubin concentrations occur in bacteremic adults (20,21). In a review of 100 consecutively enrolled adult and pediatric patients with positive results of blood cultures (4), 54% had elevated serum bilirubin levels, and 34% had values of 2.0 mg/dL or greater. The condition may be more prevalent among patients with preexisting liver disease, whereas only 6% of patients without preexisting hepatobiliary disease had jaundice in another series (22).

The molecular pathogenesis and pathophysiology of cholestasis has been reviewed (23,24). Secretion of bile depends on the adequate functioning of many membrane transport systems in both hepatocytes and cholangiocytes, and various molecular defects in hepatocellular membrane transporters are associated with cholestatic liver disease in humans. At present, ursodeoxycholic acid (ursodiol) is used in many cholestatic liver diseases, presumably because it replaces toxic hydrophobic bile salts in serum, liver, and bile. At present, however, ursodiol has no accepted role in the cholestasis of sepsis. A better understanding of the molecular mechanisms involved in cholestatic syndromes should unfold the potential for newer therapies (23).

Specific Bacterial Infections

Salmonella Hepatitis (Typhoid Fever)

Both *Salmonella typhi* and *Salmonella paratyphi* cause the acute systemic disease enteric fever. It has been estimated that 16 million cases occur per year, with at least 600,000 deaths, making typhoid fever a major public health problem in less developed regions of the world (25). Although clinical hepatitis is unusual (probably fewer than 25% of all cases), liver involvement is present in almost all cases (26).

The term *salmonella hepatitis* refers to liver injury caused by infection with either *S. typhi* or *S. paratyphi*, and the disease has been documented in both endemic and nonendemic areas. Among the 150 cases of salmonella hepatitis reported to date, most occurred in patients with typhoid infection. The disease affects people of all ages, and those with immune deficiency are particularly at risk. Among persons with human immunodeficiency virus (HIV) infection, the most commonly isolated serotypes are *Salmonella enteritidis* and *Salmonella typhimurium* (25). Alcoholism has been identified as a predisposing factor for severe forms of *S. enteritidis* infection in cases in which no other underlying disease has been evident (27,28,29).

The mechanism by which the organism causes hepatitis is not established. It may be related to either direct hepatic damage from endotoxin or the inflammatory process or to immune mechanisms. In a rodent

model of Salmonella infection, the organism invaded and multiplied extensively in hepatocytes. Thereafter destruction of infected hepatocytes by inflammatory phagocytes was followed by a release of bacteria into the extracellular space. The findings suggested that lysis of infected hepatocytes by phagocytic cells was an important early-defense strategy against liver infection with *S. typhimurium* (30). Similar mechanisms of infection occurred with *Francisella tularensis* and *Listeria monocytogenes* (see subsequent text). There is also evidence to suggest that the severity of the hepatitis in typhoid fever correlates with the virulence of the infecting organism (31,32).

The liver histology of salmonella hepatitis is nonspecific. Ballooning degeneration with vacuolation has been reported. Reticulum endoplasmic dilatation, mitochondrial alterations, and biliary canaliculus injury have been described. Occasionally, *S. typhi* organisms are found in the liver cells, as are lobular aggregates of Kupffer cells, lesions known as *typhoid nodules* (33,34). Such nodules simulate granuloma formation and represent hyperplasia of the reticuloendothelial system. This hyperplasia reportedly causes hepatic enlargement in patients with enteric fever (35).

The clinical presentation of salmonella hepatitis resembles that of viral hepatitis, but certain features help in differentiating the two diseases. In particular, high fever (often >40°C) and bradycardia (inappropriate response of heart rate to degree of fever) seem to be more common among patients with salmonella hepatitis. In addition, the biochemical profile is markedly different from that of viral hepatitis and suggests the presence of an infiltrative process rather than hepatitis. In a comparison of 27 cases of salmonella hepatitis with acute viral hepatitis, El-Newihi et al. (36) found that patients with salmonella hepatitis were more likely to have a disproportionately increased serum alkaline phosphatase level and that serum aminotransferase values were far lower than with acute viral hepatitis. Also unlike viral hepatitis, salmonella hepatitis was associated with fever and a left shift of white blood cells (37). Jaundice is unusual, and many cases of salmonella hepatitis are anicteric. In untreated patients, jaundice may be delayed appearing in the second to the fourth week of the illness. Among patients with jaundice, and presumably more severe disease, glomerulonephritis (characterized by increased blood urea nitrogen and serum creatinine levels, proteinuria, and urinary sediment red cell casts) has been more common (38,39). In the more severe cases, in addition to glomerulonephritis, complications include liver abscess, cholangitis, encephalitis or neuropsychiatric manifestations, myocarditis, or bleeding diathesis. Disseminated intravascular coagulation with extensive gangrene of the extremities and rhabdomyolysis has been described (27).

Establishing a diagnosis of salmonella hepatitis may be difficult in developing countries, because the manifestations are similar to those of other forms of acute hepatitis, including viral hepatitis, leptospirosis, and malaria. The biochemical profile described earlier helps to differentiate the various entities. The alanine aminotransferase (ALT) to lactate dehydrogenase (LDH) (ALT/LDH) ratio usually is less than 4.0 in salmonella hepatitis. A ratio greater than 5.0 is reported in acute viral hepatitis, and a ratio less than 1.5 occurs in cases of central zonal injury, such as hepatic ischemia or acetaminophen injury (36).

Prompt diagnosis and early intervention with appropriate antibiotics assure a good prognosis. Because of

salmonella resistance to the so-called first-line antibiotics (chloramphenicol, trimethoprim-sulfamethoxazole, and amoxicillin), antibiotic sensitivity testing is advised. A 5-day course of fluoroquinolone is the therapy of choice for uncomplicated enteric fever. Some experts have advocated the addition of intravenous dexamethasone (40). Because the organism can enter the bile and reside in the gallbladder, shedding for long periods can cause the chronic carrier state. Follow-up stool cultures are advised for all patients with typhoid fever to ensure that they are not carriers, and long-term fluoroquinolone therapy may help eradicate the carrier state (35). The prognosis for salmonella hepatitis is excellent; death has occurred among patients with malnutrition and immunodeficiency.

Tuberculosis

There has been a renewed interest in tuberculous infection of the liver because of the increasing incidence of extrapulmonary tuberculosis related to acquired immunodeficiency syndrome. In such cases, *Mycobacterium avium-intracellulare* is often the cause of liver dysfunction (see Chapter 51). Classically, however, hepatic tuberculosis (infection with *Mycobacterium tuberculosis*) as a part of miliary disease may occur in as many as 80% of all patients dying of pulmonary tuberculosis (41). The original description of hepatic tuberculosis classified the disease as (a) miliary, a part of generalized disease, or (b) local, with focal involvement of the liver. The terms that have led to confusion over the years included tuberculous pseudotumor, atypical hepatic tuberculosis, tuberculous cholangitis, and tuberculous liver abscess (42).

One classification scheme (42) separates hepatic tuberculosis into three categories: Miliary, granulomatous, and localized hepatic. The miliary form, which is part of generalized miliary disease, usually does not involve the liver. Granulomatous disease, that is, hepatitis due to tuberculosis, is defined by the finding of typical caseating granulomas at liver biopsy and response to appropriate antimicrobial therapy. Finally,

localized hepatic tuberculosis is further classified into disease that is either (a) without bile duct involvement (e.g., solitary or multiple hepatic nodules or tuberculous abscess) or (b) with bile duct involvement due to either compression of the bile duct by lymph nodes or actual involvement of the ductal epithelium by the tuberculous process. Therefore, the term *hepatobiliary tuberculosis* refers to a distinct clinical entity of localized hepatic disease with characteristic clinical features.

The portal of entry of *M. tuberculosis* organisms into the biliary tract and the liver is the hematogenous route or, less commonly, the portal vein or lymphatic vessels (43). Therefore disease that is isolated to the liver is considered rare, even in the absence of documented disease elsewhere; often inactive pulmonary tuberculosis is found at autopsy. One variant form of hepatic tuberculosis without active pulmonary or miliary disease is the so-called "nodular form," which presents as an isolated liver tumor or abscess (44,45). In most cases, the clinical presentation is that of neoplasm, with solitary liver lesions of variable size and imaging features, raised alkaline phosphatase values, weight loss, and so on, in the absence of known previous tuberculosis. In one recent series, PCR assay of the liver tissue in five cases established the etiologic diagnosis of *M. tuberculosis*, with postoperative histologic diagnoses only showing chronic granulomatous inflammation. Such cases underscore the difficulty in reaching the correct diagnosis of hepatic tuberculosis, the value of PCR technology in establishing this diagnosis, and the need for a high index of suspicion (44).

The clinical manifestations of hepatobiliary tuberculosis are those of the extrahepatic disease; hepatic involvement usually produces no symptoms (46). Nevertheless, cases of fulminant hepatic failure have been reported, among both immunosuppressed and immunocompetent persons (47,48). In one summary of cases of gastrointestinal tuberculosis in California, patients with hepatic involvement usually had right upper quadrant pain or fever of unknown origin (49). Nonspecific abdominal pain may be present in patients with chronic tuberculosis, whereas fever and weight loss are common in cases of tuberculous abscess. The most common physical finding is hepatomegaly, which probably occurs in most cases (46,50). A disproportionately increased serum alkaline phosphatase level is a consistent finding (51), which suggests the presence of an infiltrative hepatic process, whereas nonspecific aminotransferase elevations do not aid in diagnosis (52). The presence of jaundice suggests biliary involvement, and the biochemical profile may simulate that of extrahepatic biliary obstruction (53).

An unusual manifestation of tuberculosis involves the development of portal hypertension caused by the compression of the portal vein by tuberculous lymph nodes, followed by the rupture of esophageal varices and hematemesis (54). Isolated pancreatic tuberculosis may manifest in a manner very similar to that of a pancreatic neoplasm, including a mass lesion of the pancreatic head (55). Similarly, gallbladder tuberculosis is reportedly increasing in incidence and may manifest as biliary colic and acute cholecystitis (56).

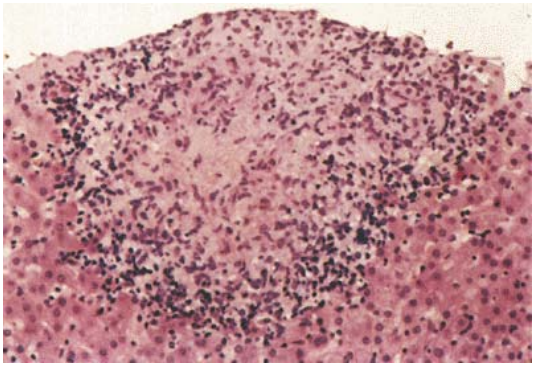
Another unusual but increasingly reported variant of hepatobiliary tuberculosis is obstructive jaundice caused by the involvement of the bile duct, pancreas, or gallbladder. Compression of the biliary tree by involved lymph nodes or possibly by direct involvement of the biliary epithelium or rupture of a caseating granuloma into the lumen of the bile duct may cause jaundice and biochemical cholestasis. Intrahepatic bile duct obstruction may result from granulomatous involvement, often as part of miliary tuberculosis. The entity of bile duct tuberculosis (57) may manifest as bile duct dilatation and common hepatic duct strictures. Biliary cytologic findings from endoscopic cholangiography may yield the diagnosis (58). Such patients have painless jaundice and weight loss that mimics malignant disease of the pancreas or cholangiocarcinoma, and dilated bile ducts are found at imaging studies. Experience with therapeutic biliary stenting has been variable, and in unsuccessful cases, percutaneous biliary drainage decompresses the obstruction (59,60).

Imaging studies may help rule out other conditions. Plain radiographs may show hepatic calcifications in patients with chronic tuberculosis, and both ultrasonography and computed tomography (CT) may show complex masses, either solitary or multiple. Such masses, common in patients with tuberculous liver abscesses, cannot be differentiated from malignant tumors and necessitate aspiration or biopsy for further investigation. One patient with cirrhotic hepatitis C and end-stage renal disease presented with multiple hyperechoic hepatic lesions on ultrasound, without pulmonary involvement (61). Blind percutaneous liver biopsy may be useful in the diagnosis of

the miliary form, whereas direct-guided biopsy with laparoscopy results in a higher diagnostic yield. At laparoscopy, a cheesy white appearance of irregular nodules, often resembling malignant tumor, has been described (42,62). Although the finding of caseating granuloma is highly suggestive of tuberculosis (Fig. 50.1), similar pathologic findings occur in brucellosis, coccidioidomycosis, and Hodgkin's disease. Caseation is commonly associated with tuberculosis, but in some series it occurs less frequently. The finding of acid-fast bacilli at biopsy occurs infrequently, fewer than 35% of cases, and the yield of a positive culture result for *M. tuberculosis* itself is even less common.

Molecular techniques establish the diagnosis of hepatic tuberculosis. Akcan et al. (63) and Alcantara-Payawal et al. (64) reported very high sensitivity (overall assay positivity of 88% in one series) with

low false-negativity among control patients, from a PCR assay on the liver tissue of patients with infection.



▪ **Figure 50.1** Tuberculosis. Caseating hepatic granuloma in a patient with fever of unknown origin.

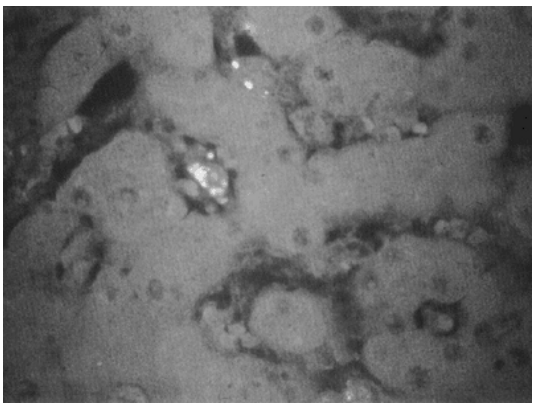
The management of hepatic tuberculosis involves the use of at least four antituberculous drugs, usually including isoniazid, rifampicin, pyrazinamide, and ethambutol (65). Although therapy traditionally lasts at least 6 months, multidrug resistant organisms require alternative chemotherapies, and there is a genuine need for new agents. It is anticipated that implementation of the recently developed molecular assays for *M. tuberculosis* will serve to assess response to therapy and allow individualized treatment duration.

Legionnaires Disease

Although most forms of pneumonia may cause derangements in liver function, Legionnaires disease, pneumonia characterized by multisystemic involvement, is particularly likely to cause abnormal results of liver tests. In one large review (66) both aminotransferase levels (up to 15 times the upper limit of normal) and alkaline phosphatase levels (up to 9 times the upper limit of normal) accompanied the pneumonia. Fifteen percent of patients also had hyperbilirubinemia. Therefore, the finding of markedly abnormal liver biochemical values in the presence of obvious pneumonia may be a clue to the appropriate diagnosis. The organism can be found with direct immunofluorescence or other techniques (Fig. 50.2) (67). Deranged liver biochemistries may represent the main manifestation (68).

Brucellosis

Three species of Brucella affect humans: *Brucella melitensis*, *Brucella abortus*, and *Brucella suis*. Brucellosis is an occupational disease that affects food handlers, and organisms enter the body through the skin or oropharynx and spread to regional lymph nodes. The disease can also be airborne and transmitted to personnel in microbiology laboratories.



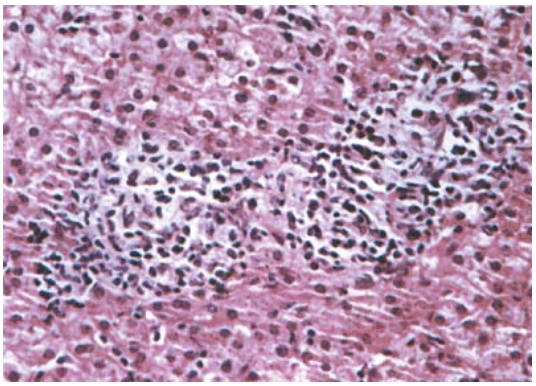
▪ **Figure 50.2** Legionella pneumophila serogroup 3. Antigenic material within sinusoidal lining cells in the liver (direct immunofluorescence, 512×). (Courtesy of John Watts, MD.)

The involvement of the liver in human brucellosis may occur with infection by both *B. melitensis* and *B. abortus*. The incidence of liver disease in brucellosis depends on the definition. Colmenero et al. (69) proposed that the concept of hepatic complication of systemic brucellosis be reserved for patients with obvious hepatic dysfunction, including jaundice or abscess. Defined in these terms, only 2.4% of 530 patients with *B. melitensis* infection had hepatic complications, whereas the presence of hepatic granuloma in the absence of overt hepatitis may be more common. In a study of 905 patients with brucellosis, Ariza et al. (70) found 16 cases of chronic hepatosplenic suppurative brucellosis (14 in the liver and 2 in the spleen) among 15 patients. One half of the patients had previous remote brucellosis. Although hepatic abscess formation after acute infection is unusual, mild nonspecific liver enzyme abnormalities may be detected in approximately 50% of patients with brucellosis. In addition to hepatic involvement, spontaneous bacterial peritonitis can occur in the absence of obvious hepatic involvement (71,72). Febrile and fatal hepatitis with hepatic abscess and endotoxic shock has been described (73).

Carazo et al. recently described the imaging features in cases of hepatosplenic brucelloma, and noted that on ultrasound, the lesion appears iso- or hypoechogenic with the liver, with focal calcifications. Contrast-enhanced CT scans showed predominantly solid masses with irregular borders, rarely with transdiaphragmatic lung invasion (74).

Histologic examination of the liver in brucellosis usually shows nonspecific portal and lobular inflammation, and small noncaseating granulomas are often associated with reactive hepatitis (Fig. 50.3). The finding of granuloma is constant when the duration of the

disease is less than 100 days but is infrequent after this time (75). The predominant biochemical derangement in clinically apparent disease is an increase in serum alkaline phosphatase level. This finding suggests the presence of an infiltrative process. The organism can be isolated from the blood in acute states, but cultures may take 3 weeks to turn positive. Agglutinating antibodies generally appear after the second week of illness, and the diagnosis usually is established by the demonstration of increasing titers. Therefore, the presence of acute infection may be established by isolation of the microorganism or by appropriate serologic findings. These diagnostic criteria may not be helpful, however, for chronic forms of the disease, which may have evolved over long periods.



▪ **Figure 50.3** *Brucella melitensis*. Nonspecific lymphoplasmacytic inflammatory infiltrate in patient with brucellosis (hematoxylin and eosin, 510×). (Courtesy of John Watts, MD.)

The more serious form of the disease therefore involves hepatic abscesses, and imaging studies show large calcium densities within the liver. Many such patients had known brucellosis many years earlier and were free of symptoms before the development of the abscess. However, even among patients without previous brucellosis, the finding of hepatic calcium deposits when the patient first arrives for evaluation strongly suggests that this entity represents a local reactivation of a previous undocumented brucellosis, as in tuberculosis (70). In the United States, the manifestation of brucellosis among children as hepatosplenic abscess may cause diagnostic confusion, particularly among immigrants and travelers from countries where brucellosis is endemic (76).

Unlike patients with acute brucellosis, patients with hepatic abscess (chronic hepatosplenic suppurative brucellosis or "brucelloma") tend not to have either leukopenia or relative lymphocytosis, and biochemical abnormalities are minor (68,75). The differential diagnosis includes neoplasm, hydatid disease, pyogenic or amebic abscess, and other granulomatous infections, including tuberculosis and histoplasmosis (77). Such patients often have had very low titers of agglutinating antibody that have delayed appropriate diagnosis.

The drugs administered for therapy for acute infection are tetracycline and rifampicin. In cases of chronic suppurative disease, percutaneous or surgical drainage should be performed (70,78).

Tularemia

Infection with *F. tularensis*, the causative agent of tularemia, occurs after exposure to jackrabbits and hares, the main animal reservoir in North America and Europe. Hunters are at risk and may acquire the disease from tick or deer-fly bites in the summer months. In Sweden, the lemming is responsible for this disease ("lemming fever"), whereas in Russia the water rat and muskrat may spread tularemia. The disorder usually affects the lungs, and the usual manifestation is a flu-like syndrome occurring between 1 and 10 days after exposure. Liver involvement is rare, and when it does occur, only modest aminotransferase abnormalities are found (79). Tularemia also manifests as obstructive jaundice with fever, suggesting the presence of cholangitis. A cholestatic biochemical profile may cause a diagnostic dilemma in such cases (80).

Early diagnosis and management of tularemia probably account for the rarity of hepatic dissemination, but hepatic tularemia may also manifest as a solitary hepatic abscess early in the course of disease (81). Histologic examination of the liver in cases of tularemia shows multiple focal areas of coagulative necrosis with a surrounding chronic inflammatory infiltrate (82). The diagnosis can be established serologically with demonstration of agglutinating antibodies. Enzyme-linked immunosorbent assay (ELISA) tests are available, but a PCR test for *F. tularensis* should enable rapid confirmation of the clinical diagnosis of tularemia (83). Treatment consists of streptomycin or gentamicin.

Listeriosis

L. monocytogenes is an organism found mostly in rodents. It is widely distributed in nature, including soil and plant material. It usually causes meningoencephalitis or pneumonitis, although hepatic involvement is reported. The liver disease of listeriosis is more common in neonates, but in adults, it may manifest as signs and symptoms of viral hepatitis, usually with high fever (84). Patients are often immunosuppressed or have underlying malignant disease (85). The onset may be gradual over several weeks or immediate fulminant hepatitis. Aminotransferase levels may be high, and jaundice may be present. The presence of high fever and leukocytosis tends to differentiate this condition from viral hepatitis, and the diagnosis is confirmed by the isolation of the organism from blood or cerebrospinal

fluid. The disease may also manifest as a liver abscess, in which case the diagnosis is established by culturing aspirated abscess material (86).

Melioidosis

Melioidosis is endemic to northeastern Thailand but is also found throughout Southeast Asia and northern Australia. It is a potentially fatal infection caused by the bacterium *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*). The organism is ubiquitous in many parts of the tropics, and is found in damp soil and freshwater. The usual mode of acquisition is through inhalation or through minor skin abrasions. The mortality may be high, averaging 45%, and in cases of acute septicemia, death occurs within the first 3 days after hospital admission. The disease has protean manifestations, and histopathologically it mimics tuberculosis and catscratch disease. Early diagnosis and proper antibiotic therapy (parenteral ceftazidime was provided for 4 weeks in the report of Ben et al. (87)) are crucial.

Melioidosis may present with pulmonary infection, tonsillitis, localized abscess or fulminant septicemia (87). Liver involvement in melioidosis is common, but the increase in aminotransferase and serum bilirubin values is similar to that among patients with other forms of bacteremia. Visceral organ abscesses are common and usually involve the spleen, liver, and kidney. Granulomas may be found. The sonographic appearance of these multiple, small, discrete abscesses is target-like, and larger multiloculated abscesses are common (88). The differential diagnosis of melioidosis includes other forms of liver abscess, tuberculosis, and other bacterial causes of sepsis. Immunohistochemistry plays a useful diagnostic role, and polyclonal antibodies applied to formalin-fixed, paraffin-embedded tissue help establish the diagnosis earlier than the traditional but less reliable culture techniques (89).

Spirochetal Infections

Leptospirosis

Professor Weil of Heidelberg first made the classic description of febrile headache, jaundice with renal failure, and severe muscle pain, which is now known as *Weil disease*. Yet only a small percentage of patients infected by spirochetes of the genus *Leptospira* have this most severe of manifestations, and most descriptions of liver pathology in this disease come from autopsy series. Early recognition, with administration of appropriate antibiotics, has resulted in a very low incidence of hepatic manifestations.

Leptospirosis has a worldwide distribution. It results from direct or indirect exposure to the urine of infected animals, usually rodents. The most common serotypes are *Leptospira icterohaemorrhagiae*, carried by rats, and *Leptospira canicola*, carried by dogs. The organism enters the body through wounds on the skin and through intact mucous membranes and can directly penetrate the skin. Therefore the disease often results from occupational exposure, as among persons who work in sewers, mines, and construction sites and among food workers who may be exposed to rodent-infested environments. Leisure activities that include swimming, white-water rafting, or fishing in rivers or ponds contaminated with infected urine from wild animals also have resulted in leptospiral infection (90). In Thailand, risk factors include walking through water, applying fertilizer or plowing wet fields (91). Eating uncooked rice has been reported as a risk factor because of contamination with rat urine (90).

Although usually considered a disease of developing nations, cases occur in the United States and may cause a diagnostic dilemma. Among immunosuppressed HIV-infected patients, homelessness in large cities may result in exposure to rodent urine and resultant urban leptospirosis (92). The usual manifestations are acute fever and a

flu-like illness, often with cough and chest pain, that occur after a 7- to 10-day incubation period. The abdominal pain of the acute phase may simulate surgical abdomen and may manifest as biliary colic. At a recent triathlon in Illinois, which included swimming in a freshwater lake, two athletes had clinically suspected acute cholecystitis and underwent cholecystectomy. An immunohistochemical test for leptospirosis applied to these gallbladders showed bacterial antigens and intact bacteria (93). Central nervous system symptoms of headaches and confusion may occur in the acute stage.

A second phase occurs during the second week of illness. The patient may have a milder recurrence of the aforementioned symptoms. This is the so-called icteric stage and may be caused by the effects of an activated immune system (90,94). Muscle pain may become severe, especially in the lower extremities. Marked conjunctival congestion (suffusion) occurs within the first few days of the illness and may persist into the second stage. Jaundice may or may not be present at this stage. Renal function worsens during this second phase, and a progressive increase in serum creatine kinase level reflects the presence of myositis.

The third, or convalescent, stage starts in the third week with progressive improvement in mental status and renal function and relief from jaundice. The so-called classic Weil disease is actually a recurrence of the fever after termination of the first stage of the illness, and the initial biphasic course is bypassed. Hepatic involvement is usually self-limited, and "microcirculatory abnormalities" (95) have been implicated as a cause of high bilirubin values. One recent report from Japan (96) described a case of simultaneous hepatitis E

infection and leptospirosis, with prolonged cholestasis and jaundice lasting several months.

Doxycycline may be effective prophylaxis of leptospirosis (97). Once the disease has developed, early intensive care and administration of doxycycline may be lifesaving (98), and therefore early diagnosis is important. During the first phase, leptospires can be isolated from the cerebrospinal fluid and blood; in the second phase, they are isolated from the urine. Dark-ground microscopic examination of plasma has been found to be a simple and rapid form of early diagnosis of leptospirosis with hepatorenal involvement (99). PCR based on the *flaB* gene of *Leptospira* has been found to be an efficient tool for the rapid detection and identification of *Leptospira* from clinical specimens (100).

Syphilis

Syphilis is a multisystemic disease caused by the spirochete *Treponema pallidum* subsp. *pallidum*, and is a microaerophilic gram-negative bacterium. Like tuberculosis, syphilis, once a disease affecting primarily homosexual men, has been increasingly reported among heterosexuals in the urban areas of the United States. The disease spans several stages, from congenital involvement to tertiary disease, and derangements in liver function may occur in all stages. In the congenital form of the disease, liver manifestations generally occur between the ages of 2 and 15 years, with hepatic gummas. Biochemical hepatitis with jaundice may occur, and therefore a wide differential diagnosis can produce a diagnostic dilemma. A positive result of an ELISA test for immunoglobulin G (IgG) antibodies against treponemal antigen and the fluorescent treponemal antibody (FTA)-immunoglobulin M (IgM) help establish the correct diagnosis.

In a 1917 paper on the subject (101), jaundice was reported to occur in as many as 12% of cases of secondary syphilis, probably owing to inflammation of hepatocytes. The pathologic findings generally include lymphocytic and neutrophilic infiltrates in the portal tracts; pericholangiolar inflammation has also been described. Spirochetes are infrequently seen (10% in one series); identification of treponemes in the liver is even less common. Therefore, direct hepatotoxicity by the organism is probably a less likely pathogenesis of hepatitis than are immune-mediated mechanisms. Invasion of the portal venous system through the rectal portal entry in homosexual men with primary anal or rectal lesions may explain a reportedly higher frequency of syphilitic hepatitis in this population.

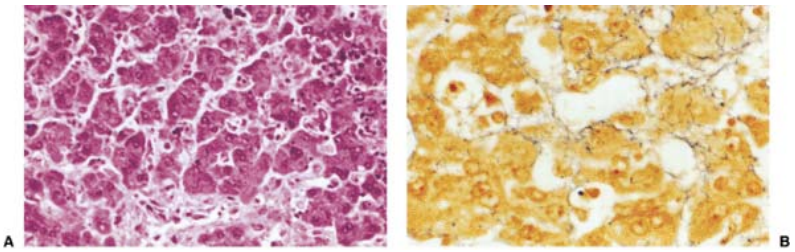
The clinical manifestations may be subtle in an anicteric patient, who may have anorexia, weight loss, and hepatomegaly. The initial signs and symptoms may include pruritus and proteinuria (102,103). The rash of secondary syphilis usually is present, whereas the primary chancre usually is no longer present. In the absence of jaundice, biochemical cholestasis with a disproportionately increased serum alkaline phosphatase level may provide a clue to the diagnosis (104,105,106). Syphilitic hepatitis may coexist with nephrotic syndrome due to syphilitic membranous glomerulonephritis (107). However, because many of the reported cases of presumed syphilitic hepatitis occurred before the advent of viral hepatitis serologic tests, Veeravahu (105) suggested that the evidence to implicate *T. pallidum* as a liver pathogen in early syphilis is not convincing. Nevertheless, the occurrence of acute cholestatic syphilitic hepatitis in the era of viral hepatitis testing has been described (108).

Additional laboratory studies of the syphilitic hepatitis of secondary-stage disease include a hemagglutination test for *T. pallidum* and an FTA absorption (FTA-ABS) test. Liver imaging studies may reveal focal liver lesions as large as 3 cm in diameter. In an unusual case from France, Maincent et al. (109) reported a case of tertiary hepatic syphilis that manifested as multinodular hepatic metastasis. This case shows that the entity of hepatic syphilis may manifest in a variety of misleading ways.

Liver involvement in cases of tertiary syphilis usually is discovered at the postmortem examination. The gummas of the liver may resemble metastatic disease at autopsy or may resemble cirrhosis because of the nodular configuration of the liver in the later stages. Hepar lobatum refers to the lobulation of the liver because it appears to be divided into several smaller lobes by deep furrows. The lobulation originates from the resorption of gummas in the tertiary stages of the disease (110). Focal liver lesions with filling defects on CT scans may similarly mimic metastatic disease in a patient with weight loss. Diagnostic liver biopsy is therefore essential (109) (Fig. 50.4). Appropriate intervention with proper antimicrobial therapy may reduce the size of the liver lesions.

The incidence of primary and secondary syphilis, as noted in the preceding text, has increased in the United States in recent years, especially among HIV-positive individuals (111). Regarding the involvement of the liver, the last large review of syphilitic hepatitis that was described in the medical literature was 30 years ago (112)

until the recent report of seven such cases among HIV-infected patients (113). These individuals presented with rash and predominant alkaline phosphatase elevations, with symptomatic and biochemical improvement following antibiotic therapy. The authors noted that high rapid plasma reagin (RPR) titers were more likely to be present if $CD4^+$ cell counts were higher. These cases emphasize the importance of entertaining the diagnosis of syphilitic hepatitis as a cause of otherwise unexplained high alkaline phosphatase levels among HIV-infected patients.



▪ **Figure 50.4** Hepar lobatum. **A:** Diffuse pericellular sinusoidal fibrosis in congenital syphilis of liver (hematoxylin and eosin, 485×). **B:** Numerous spirochetes layered in periphery of sinusoids adjacent to hepatocyte cords (Dieterle, 1,200×). (Courtesy of John Watts, MD.)

Lyme Disease

One of the less recognized manifestations of multisystemic Lyme disease is hepatitis, yet hepatic involvement appears to be common. It has been suggested that Lyme disease resembles syphilis, in that a spirochete organism causes both diseases (in the case of Lyme disease, a zoonosis, the tick-borne *Borrelia burgdorferi*), and both diseases may develop as an acute or chronic multisystemic inflammatory disease (114). The mechanism by which liver injury occurs is not known. Direct invasion of the liver by the organism and immune-complex deposition have been proposed. In one case of Lyme disease–related hepatitis in a human, the organism was found in the liver with Dieterle staining, and the patient's condition improved with doxycycline therapy. This result suggests that borrelial invasion may cause direct hepatocyte damage (115).

Lyme disease has both an early, acute stage and a chronic phase (116). Involvement of the liver is more common in the early stage. Histologic examination shows portal inflammation, ballooning of hepatocytes, considerable mitotic activity, hyperplasia of Kupffer cells, prominent microvesicular fat, and sinusoidal mononuclear and neutrophil cell infiltration (115). Clinically, in addition to other features of erythema chronicum migrans, hepatosplenomegaly and biochemical hepatitis may persist for several weeks. The acute phase may manifest as a febrile illness with jaundice and mixed hepatitic and cholestatic abnormalities (117).

In a review of the cases of 115 patients with erythema migrans, the characteristic rash of early Lyme disease, approximately one third of the patients were found to have abnormal serum ALT values. Among those with early disseminated Lyme disease, two thirds of the patients had abnormal liver biochemical values (118). The investigators concluded that liver function test abnormalities are common among patients with erythema migrans but that these abnormalities are generally mild and improve with antibiotic therapy. Zaidi and Singer recently reviewed biochemical abnormalities of the liver in patients with Lyme disease (118a).

Rickettsial Infections

Q Fever

The causative rickettsia of Q fever is *Coxiella burnetii*. It was first described in Australia in the 1930s after an outbreak of an undiagnosed febrile illness among workers at an abattoir in Brisbane; the Q represents query. The source of infection is infected sheep, goats, or cattle, and the infection may be transmitted through contact with unpasteurized milk or contact with livestock. The organism has been identified in ticks, and the disease has a worldwide distribution. Liver involvement is common.

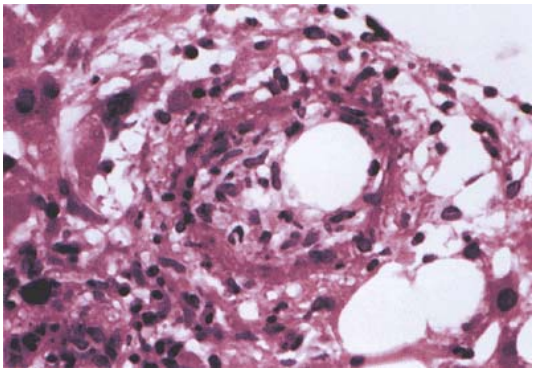
The typical presentation of Q fever is a febrile, flu-like illness with pneumonitis. The usual epidemiologic risk factors of exposure to sheep, cattle, and goats may be lacking in many cases, and often the disease goes undiagnosed (119). Hepatitis is common (120), and the disease may manifest as hepatitis in the absence of pulmonary manifestations (121). In a report of 63 sporadic cases of Q fever in an urban adult population in Spain (122), approximately 50% of patients had accompanying hepatitis.

Three main hepatic manifestations of Q fever have been proposed: A clinically acute hepatitis-like illness without respiratory involvement (the most common form of hepatic involvement); an incidental finding of increased liver biochemical values in a patient with known acute Q fever; or fever of unknown origin with characteristic hepatic granulomas (123,124,125). After an incubation period of 14 to 26 days, patients have a fever and flu-like symptoms, often with a dry cough.

Bradycardia may be present. Liver biochemical abnormalities are common but nonspecific and may manifest as anicteric hepatitis. In an appropriate setting, the disease mimics several other conditions, including other fungal infections of the liver, other granulomatous diseases, drug reactions, and so on. A 70-year-old man with cancer presented with low-grade fever and fatigue following a vacation in the Canary Islands, an area endemic for Q fever. His illness progressed to jaundice and fatal liver failure; the following year, after considering the diagnosis, an indirect microimmunofluorescence test for *C. burnetii* IgG and IgM antibodies were strongly positive, therefore confirming acute Q fever (126).

The classic doughnut granuloma of Q fever—a central clear space in the center of the granuloma—is not pathognomonic for the disease and may be seen in Hodgkin's disease and infectious mononucleosis (Fig. 50.5). The granulomas have been shown to disappear over a period of 3 months after appropriate antibiotic therapy (127). The granuloma is a dense fibrin ring surrounded by a central lipid vacuole and is composed of neutrophils, monocytes, eosinophils, and occasional multinucleated giant cells. Kupffer cells are hypertrophied and there may be a lymphocytic portal inflammation with erosion of the limiting plate (128).

The disease may be prolonged, may affect children exposed to farm animals (129), and one third of patients may have jaundice. The diagnosis is established when an increase in complement fixation is detected or the result of an immunofluorescent antibody titer to *C. burnetii* is positive (123). PCR can be used to amplify *C. burnetii* DNA from tissue (130,131). Tetracycline is considered the treatment of choice, although the less effective erythromycin may be an appropriate alternative in the treatment of children. A recently developed vaccine may be efficacious in persons at high risk (132).



▪ **Figure 50.5** Section shows classic doughnut granuloma. The characteristic lesion of Q fever is a doughnut granuloma similar to that shown here (hematoxylin and eosin, 780×). (Courtesy of John Watts, MD.)

Ehrlichiosis

Ehrlichiosis is a rickettsial infection that occurs in animals and humans, and is caused by microorganisms of the genus *Ehrlichia*. The pathogenesis and comparative pathology and immunohistology of ehrlichiosis have been reviewed (133). Human infection with *Ehrlichia canis*, a tick-borne infection common among dogs, was first reported in 1987. Recent molecular characterization, however, has suggested that taxonomic reorganization will more accurately define the Ehrlichia species. Therefore, *Ehrlichia chaffeensis* causes human granulocytic ehrlichiosis, and the major tick vector is a member of the genus *Ixodes* (134).

Involvement of the liver in human ehrlichiosis is largely anecdotal, but it can be a multisystemic disease with intense cholestasis. In one case following a documented tick bite, a 56-year-old man had multisystemic disease with sepsis and renal failure complicated by deep jaundice. Liver biopsy showed intense bile stasis and intense neutrophilic infiltration of bile ducts that suggested extrahepatic bile duct obstruction with cholangitis. A rickettsial immunofluorescent antibody panel confirmed the presence of ehrlichiosis, and the patient responded to a course of chloramphenicol (135). A tetracycline such as doxycycline also is reported to be effective.

In a review of eight cases of ehrlichiosis managed at an Arkansas medical center, seven patients had raised aminotransferase levels that suggested biochemical hepatitis, and three patients had jaundice with a peak bilirubin level of 13.8 mg/dL. All eight patients were treated with and responded to doxycycline, including one patient who had multiple-organ failure but eventually recovered. The authors concluded that in the appropriate clinical setting, ehrlichiosis should be considered a cause of elevated liver enzyme values (136).

Rocky Mountain Spotted Fever

Infection with the tick-borne *Rickettsia rickettsii* causes the multisystemic disease that is occasionally associated with an increased alkaline phosphatase level and jaundice (137). Zaidi and Singer recently reviewed the gastrointestinal and hepatic manifestation of Rocky Mountain Spotted Fever (118a).

Tick-Borne Diseases

The prevalence of tick-borne diseases has been increasing in the United States as a result of greater outdoor activity and migration of the population into rural areas (118a).

The eight most common tick-borne diseases include Lyme disease, ehrlichiosis, Rocky Mountain spotted fever, tularemia, Colorado tick fever, tick-borne relapsing fever, Q fever, and babesiosis; many of these entities have been considered separately in this chapter. Table 50.1 summarizes the gastrointestinal and hepatic manifestations of tick-borne diseases. With the exception of Colorado tick fever and babesiosis, most of these infections may cause a form of acute hepatitis and should be considered in areas of endemicity. Ehrlichiosis is most likely to cause cholestasis and jaundice, whereas Q fever, Lyme disease, ehrlichiosis and, to a lesser extent, tularemia, may cause granulomatous hepatitis.

Table 50.1. Laboratory and Clinical Manifestations of Tick-Borne Liver Infections

Manifestation	Lyme disease	Ehrlichiosis	RMSF	Tularemia	Colorado tick fever	TBRF	Q fever	Babesiosis
Anorexia	+	++	+	+	+	+	+	+
Nausea	+	++	++	++	++	+++	++	+
Vomiting	+	++	++	++	++	+++	++	+
Abdominal pain	+	++	++ to +++	++	+	++	+	+
Diarrhea	+	++	++	++ to +++	+	+ to ++	++	+
Hepatomegaly	R	+ to ++	+	+ to ++	R	+	+	+
Splenomegaly	+	+ to ++	+	+ to ++	R	R to +	+	+
Jaundice	+	+++	+	+	+	+	+	+ to ++
Elevated bilirubin level	+	+++	+ to ++	+	+	+	+ to ++	++ to +++
Elevated ALT level	++	++++	++ to +++	++	+	++	++[a]	+

[a]Elevated alkaline phosphatase level is the predominant abnormality.
ALT, alanine aminotransferase; R, rare; RMSF, rocky mountain spotted fever; TBRF, tickborne relapsing fever; +, uncommon; ++ common; +++, very common; ++++, almost always present.
Reprinted with permission from Zaidi SA, Singer C. Gastrointestinal and hepatic manifestations of tick-borne diseases in the United States. *Clin Infect Dis* 2002;34:1206–1212.

Fungal Infections

Histoplasmosis

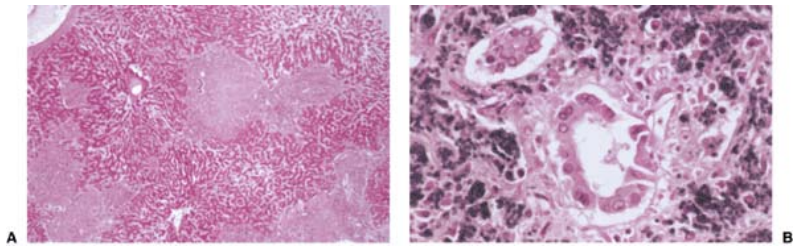
Histoplasmosis has a worldwide distribution, including the central and northeastern United States, Central and South America, India, and the Far East. It is the most common cause of fungal infection in the Ohio River Valley of the United States. It is usually transmitted after inhalation of the organism *Histoplasma capsulatum*, which is particularly associated with bird droppings, especially those of chickens. In most cases, exposure to the fungus is asymptomatic, and documentation of previous exposure is in the form of delayed-type cutaneous sensitization.

Although liver involvement is common in cases of disseminated histoplasmosis, in which case the infection may travel from the lungs to involve other organs, the disease may also present as an isolated liver mass or as an infiltrative liver disorder. A 39-year-old HIV-negative alcoholic man from New Delhi presented with a 3-month history of weakness associated with fever and jaundice. Laboratory studies showed anemia, leukocytosis, and marked alkaline phosphatase elevation. A pharyngeal culture was positive for *H. capsulatum*, and a liver biopsy

showed granulomas consisting of macrophages and giant cells. Multiple periodic acid-Schiff (PAS) positive ovoid fungal bodies were seen causing a swelling of the Kupffer cells consistent with the diagnosis of hepatic histoplasmosis, and treatment with amphotericin B was started with clinical improvement. This case, with a negative chest x-ray, demonstrates the potential for isolated liver involvement in cases of histoplasmosis (138).

Hepatic involvement with histoplasmosis may manifest as fever of unknown origin and cause a considerable diagnostic dilemma (139). It may also manifest as unexplained biochemical cholestasis with fatigue and weight loss, which can mimic neoplasia or even cholangitis (140). The disease occurs among persons without HIV infection, but immunosuppression in the form of chronic glucocorticoid therapy may be present. Liver biochemical abnormalities in many cases may be nonspecific, and biopsy may be needed for the correct diagnosis. Thrombocytopenia may be present and necessitate a transjugular approach to biopsy. Hepatic lesions may include diffuse granulomas distributed throughout the liver or parenchymal infiltration with macrophages filled with the organism, seen with fungal staining. One report described a rare case that presented as a solitary right-sided liver lesion invading the diaphragm (141).

A review of the pathologic spectrum of cases of gastrointestinal histoplasmosis showed that 10% of 52 patients had histologic evidence of liver disease, most commonly portal lymphohistiocytic inflammation (Fig. 50.6). Discrete hepatic granulomas were found in fewer than 20% of livers that were involved (142).



▪ **Figure 50.6** Elderly diabetic man who lived in an area endemic for histoplasmosis had fever and hepatomegaly. **A:** Confluent portal granulomas in a patient with hepatic histoplasmosis (hematoxylin and eosin). **B:** Special stain shows uniform, oval, yeast-like cells morphologically typical of *Histoplasma capsulatum* (hematoxylin and eosin, methenamine silver). (Courtesy of Laura Lamps, MD.)

Early diagnosis may be lifesaving, so this infection requires diagnostic consideration. Management is the same as that of other disseminated fungal infections and usually includes intravenous amphotericin B. The use of newer antifungal agents has not been described.

Another case of an unusual manifestation of hepatic histoplasmosis involved a 56-year-old "university lecturer" from Canada with a 10-year history of disabling fatigue, and a 6-month history of anorexia, weight loss and abnormal liver biochemistries, primarily alkaline phosphatase elevations. A liver biopsy showed non-caseating granulomas with multinucleated giant cells, and screening serologies were positive for *H. capsulatum*. His only risk factor was having lived in Indiana from his youth. Additional studies confirmed Addisonian crisis. After appropriate antifungal therapy, his fatigue and constitutional symptoms improved (143).

Candidiasis

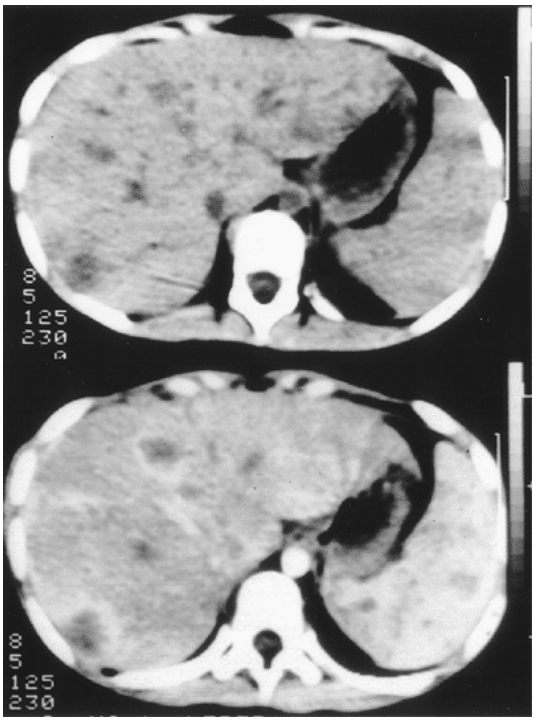
Caused by infection with the fungi of the genus *Candida*, the clinical spectrum of candidal liver disease is varied. The most common species causing human infection is *Candida albicans*, although other species also cause disease. Liver involvement in systemic candidiasis often goes unrecognized, and hepatic lesions may be found incidentally at autopsy. Therefore the entity of focal hepatic candidiasis may be part of the syndrome of hepatosplenic candidiasis or, more appropriately, chronic disseminated candidiasis (144), because other organ systems may be involved. Although systemic disease is presumed, results of blood cultures may be negative, and appropriate diagnosis requires a high degree of clinical suspicion.

The typical patient has leukemia and fever, jaundice, and biochemical cholestasis after induction chemotherapy–associated neutropenia. After the nadir of neutropenia, the serum alkaline phosphatase level begins to increase, suggesting the presence of an infiltrative or infectious process. The pathogenesis of the disease probably relates to mucosal damage of the colonic mucosa at the time of neutropenia. Local invasion and subsequent entry of the Candida organisms into the portal circulation result in liver infection (145). Results at CT and ultrasonography are often normal in the early stage of neutropenic fever, whereas as the neutrophil count returns to normal, imaging studies may show focal liver lesions with a bull's-eye appearance (145,146,147,148) (Fig. 50.7). These lesions may be absent on images, however, even with jaundice. In such cases, diagnostic laparoscopy with local anesthesia may show discrete focal yellowish-white punctate lesions scattered throughout the liver surface (149) (Fig. 50.8). Direct-guided biopsy (Figs. 50.9, 50.10 and 50.11) may establish the diagnosis and allow for appropriate antifungal therapy.

Cholangitis with common bile duct stenosis secondary to *Candida* colonization of the biliary tract was recently

described in a patient on long-time mechanical ventilation. Cholangiography revealed bead-like deformity consistent with sclerosing cholangitis. Microbiologic analysis of aspirated bile confirmed Candidiasis (150).

Although the diagnosis of hepatic candidiasis required histologic findings previously, confirmation by culture (not possible in formalin-fixed samples), or immunofluorescence, or PCR testing now allows for a sensitive and specific diagnosis, and further permits *Candida* species identification. Kirby et al. (151) described a typical case of hepatosplenic candidiasis in which PCR was positive for candida DNA in both the sera and liver biopsy. The authors noted the importance of identifying species, because *candida* shows species-specific antifungal resistance patterns, that is, some resistant to fluconazole and others to amphotericin.



▪ **Figure 50.7** Computed tomography scan of an 8-year-old girl with leukemia undergoing chemotherapy who had a fever. Images before (top) and after (bottom) administration of contrast material reveal numerous areas of low attenuation throughout the liver and spleen. Rim enhancement of the lesions after injection of contrast material is caused by inflammation. Open biopsy of the hepatic lesion proved the diagnosis of hepatic candidiasis. (Courtesy of Ali Shirkhoda, MD.)

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Optimal therapy for hepatic candidiasis has not been established, in part because of the rarity of the condition, the paucity of controlled trials, and the absence of established end points of treatment. A relapse of the infection may be related to either premature discontinuation of therapy or inadequate antifungal treatment of patients with chemotherapy-induced neutropenia (144). Furthermore, the hepatic lesions of chronic disseminated candidiasis may transiently disappear during neutropenia, and therefore antifungal therapy should not be discontinued on the basis of radiologic findings alone (152). The original therapy for this infection consisted of amphotericin B, yet prolonged treatment with amphotericin B may cause renal toxicity and often fails to eradicate infection. Some experts have advocated the addition of other antifungal agents or the addition of liposomal formulations (153,154), which may be better targeted to enter the liver. Pappas et al. recently reviewed the treatment of candidiasis (155). Both fluconazole and caspofungin are as effective as and less toxic than amphotericin B, and can

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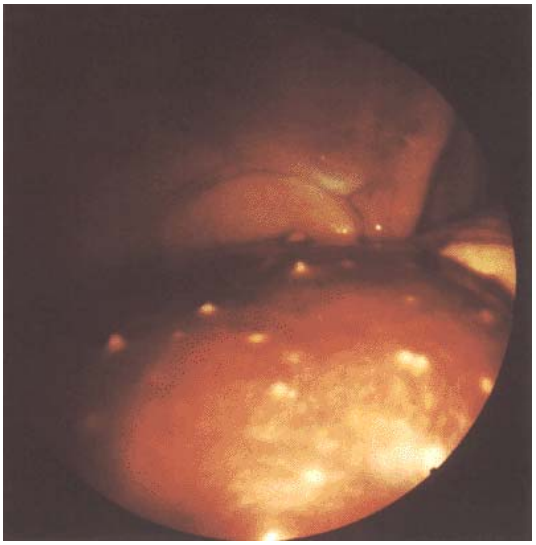
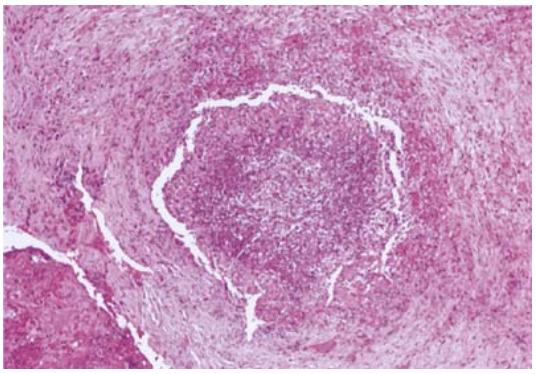
be given orally (153,156). As illustrated in the recent case report by Kirby et al. (151), identification of the specific *candida* species may guide appropriate therapy, such as an oral azole, therefore obviating the need for prolonged parenteral amphotericin treatment. The heightened awareness of the entity of hepatic candidiasis in the patient with neutropenic leukemia undergoing chemotherapy has highlighted the importance of factors that promote its development, including intravenous catheters and broad-spectrum antibiotics; furthermore, there has been the suggestion that prophylactic antifungal agents may prevent systemic fungal disease, but results are conflicting (157,158).



▪ **Figure 50.8** A 60-year-old woman with leukemia had fever and jaundice after induction chemotherapy. Computed tomography scan shows no significant pathologic process. At laparoscopy, however, the liver was diffusely infiltrated with small discrete lesions of focal candidiasis.

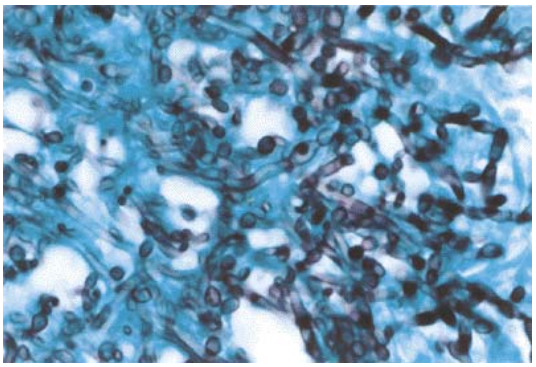


▪ **Figure 50.9** Focal hepatic candidiasis. Same patient as in Fig. 50.8 is shown here. Centrally necrotic granuloma is surrounded by a thick fibrous capsule (hematoxylin and eosin, 80×). (Courtesy of John Watts, MD.)

▪ **Figure 50.10** Focal hepatic candidiasis. Gross wedge biopsy specimen of liver shows necrotic granuloma encapsulated by fibrous tissue with central cavitation. This cavitation results in the bull's-eye lesion often seen on imaging studies. (Courtesy of John Watts, MD.)

Actinomycosis

Actinomycosis is a chronic, progressive, suppurative disease caused by actinomycetes of the genus *Actinomyces*, notably *Actinomyces israelii*, *Actinomyces bovis*, and *Actinomyces naeslundii*. Infection often occurs in the mouth (cervicofacial) area, thorax, abdomen, or uterus (pelvic). Early management of dental sepsis is believed to have prevented the cervicofacial form of the disease, which is thought to cause infection by local proliferation of organisms (159).



▪ **Figure 50.11** Focal hepatic candidiasis. Grocott-stained section shows numerous yeast-like cells and mycelial elements of *Candida* in the center of a granuloma (80×). (Courtesy of John Watts, MD.)

The involvement of the liver is rare, but primary hepatic actinomycosis is an important differential diagnosis to hepatocellular carcinoma in endemic areas, and may present as a solid liver mass. In a recent review of 57 cases reported in the literature, the mean age of patients was 43 years (range, 4–65 years) with a male predominance. Most patients presented with fever, abdominal pain and weight loss, typically with a subacute presentation of up to 18 months. Leukocytosis was common, as was serum alkaline phosphatase elevations (160).

Hepatic actinomycosis had been reported to occur in 15% of abdominal cases (5% of all cases). In a review of 11 cases of actinomycosis of the liver from Japan (161), investigators found that in six cases (55%), partial hepatectomy had been performed because of involvement of liver tumors and that five patients had liver abscess. The authors concluded that hepatic actinomycosis should be considered in the differential diagnosis of pyogenic liver abscess and space-occupying lesions of the liver.

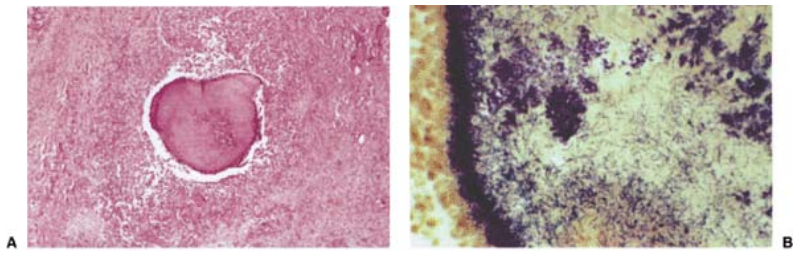
The hepatic disease probably is caused by spread through the portal vein caused by a mucosal injury due to ulcer, inflammatory bowel disease, or surgery. Local aggregates of Actinomyces organisms are often associated with other bacteria, such as coliforms, and these other bacteria may be involved in the pathogenesis of the infection. The hallmark of the infection is the formation of inflammatory masses containing granules (Fig. 50.12) (162,163).

The CT scan appearance may be a multiloculated low-attenuation lesion with low intensity on the T1-weighted sequence at magnetic resonance imaging (high intensity on the T2-weighted sequence) with surrounding edema. A peripherally thickened and irregular inner wall may suggest the presence of an abscess (164). Angiography may show a hypervascular hepatic mass mimicking hepatic neoplasm in the arterial phase (166,167). These radiographic features are nonspecific, however, and there is debate regarding how best to establish the diagnosis.

Establishment of the correct diagnosis may be prolonged, as noted in the preceding text, because cases may

manifest as nonspecific subacute presentations including fever of unknown origin (162,165) or inflammatory pseudotumors (163), which have the gross appearance of malignant lesions. This manifestation emphasizes the need to search for bacteria in such lesions. Often such patients have underlying sepsis due to chronic abdominal abscesses. The histologic examination of tissue samples (168) is needed to establish the appropriate diagnosis. In one case (169) a positive blood culture established the correct diagnosis, but a recent review of hepatic actinomycosis (170) observes that percutaneous or surgical interventions for tissue samples were more likely to be diagnostic than

positive peripheral blood cultures for *A. israelii*. In rare instances, the lesion may infiltrate the diaphragm and right lung (162).



▪ **Figure 50.12 A:** Microcolony of Actinomyces organisms (sulfur granule) surrounded by acute suppurative inflammation (hematoxylin and eosin, 80×). **B:** High-power Brown-Brenn stained section of a sulfur granule, which is composed of gram-positive filamentous bacteria. The diagnosis of actinomycosis was confirmed with direct immunofluorescence (not shown) (780×). (Courtesy of John Watts, MD.)

Most patients respond to prolonged intravenous penicillin G, alone or in combination with clindamycin or ciprofloxin, often for several months (160). Lack of response to such therapy may necessitate surgical drainage. Alternative regimens include erythromycin and tetracycline. One recent report described a 53-year-old immunocompetent male with hepatic actinomycosis who failed to respond to intravenous antibiotic and underwent right posterior hepatic segmentectomy, with successful resolution of infection and without evidence of recurrence (171).

Coccidioidomycosis

The disease known as *San Joaquin Valley fever* is caused by the dimorphic fungus *Coccidioides immitis*. It is endemic in the Southwest region of the United States, Central America, and Mexico and is characterized by a respiratory infection and fever after an incubation period of 7 to 28 days. The route of infection follows inhalation of the fungus, and the disease may then spread from the primary lung focus to involve the liver. Extrapulmonary disease is rare, but case reports clearly show that both liver and biliary involvements are manifestations of coccidioidomycosis.

A 26-year-old man from New Delhi had right upper quadrant pain, and ultrasonography revealed a solitary right lobe liver abscess. Aspiration of the abscess revealed pure growth of coccidioidomycosis, which was confirmed with culture (172). In the United States, 8 of 1,347 (0.59%) patients who underwent liver transplantation at a California medical center had coccidioidomycosis, showing that this organism can cause a serious and in some cases fatal infection after liver transplantation and that the incidence of this disease appears to be increasing (173).

The usual clinical features are those of nonspecific anicteric hepatitis, often with biochemical cholestasis, in a person who has recently traveled to Mexico or the American Southwest. It commonly presents as a hepatopulmonary syndrome with eosinophilia (174). Biopsy of the liver shows granulomatous hepatitis (174,175). In rare instances, obstructive jaundice may relate to granulomatous involvement of the bile duct epithelium. Ramirez et al. (176) reported the case of a 43-year-old man from Arizona who had fever and abdominal pain followed by jaundice and a cholestatic biochemical profile with no obvious lung involvement. The bilirubin level reached 7.4 mg/dL, and endoscopic cholangiography showed an irregular stricture involving the common bile duct and intrahepatic biliary tree necessitating placement of a biliary stent. Biopsy of the lymph node performed at laparotomy showed granuloma with spherules, and complement fixation antibody testing confirmed the presence of coccidioidomycosis. Endoscopic retrograde cholangiopancreatography after successful therapy (fluconazole and intravenous amphotericin B) showed resolution of the stricture.

Among 37 immunosuppressed patients who received liver transplants who later moved to Arizona, the incidence of new coccidioidal infection was 2.7%, suggesting that coccidioidomycosis was not frequent in this population (177). Nevertheless, among patients with end-stage liver disease listed for transplantation in this same region of Arizona, the incidence of new coccidioidal infection was 4.2%, compared with 0.04% in the same county in the general population. The authors suggested that treatment might alleviate some of the

symptoms of coccidioidomycosis originally attributed to disease of the liver (178).

Other Infections

Neisseria

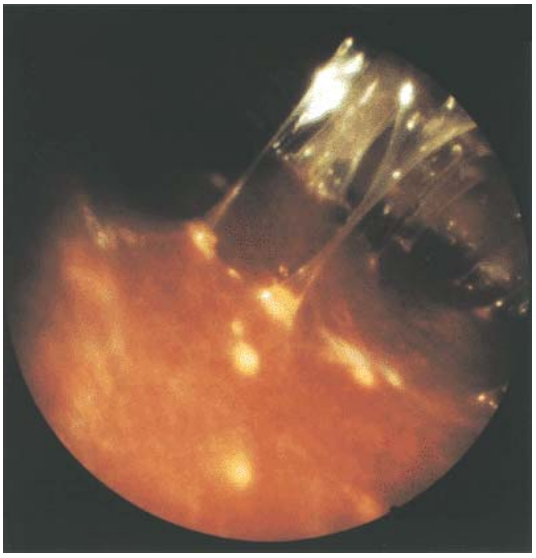
Nonspecific aminotransferase abnormalities occur in disseminated gonococcal infection (179,180). Cervical and pelvic gonorrhea may be associated with violin-string adhesions between the liver capsule and the peritoneal wall, known as Fitz-Hugh-Curtis syndrome. However, such adhesions are not pathognomonic for Neisseria infection, and may occur after other infections, including hepatic candidiasis (Fig. 50.13) (149).

Chlamydia

A similar syndrome of perihepatitis occurs with infection with *Chlamydia trachomatis* (181), which may also cause prolonged fever and liver granuloma (182).

Campylobacter

Mild liver dysfunction as well as acute hepatitis–like biochemical values may occur after infection with *Campylobacter* organisms (183). The organism has been isolated from bile during episodes of cholecystitis in association with gallstones (184).



▪ **Figure 50.13** Perihepatic adhesion (Fitz-Hugh-Curtis syndrome) may occur in conditions other than gonorrhea. After therapy for hepatic candidiasis, a follow-up laparoscopy showed violin-string adhesions from the focal lesions to the peritoneal wall. Such adhesions may result in marked right upper quadrant pain.

Shigella

Anecdotal case reports include a case of cholestatic hepatitis following infection with *Shigella sonnei* (185) and anicteric hepatitis associated with *Shigella flexneri* infection (186).

Yersinia

Cases of granulomatous hepatitis and cholestasis have been reported in association with disseminated yersinia infections (187).

Catscratch Disease

Hepatosplenic catscratch disease often manifests as fever of unknown origin in children who have had contact with an immature cat. The disease occurs when *Bartonella henselae* causes necrotizing granuloma in the liver or spleen or both. Fleas have also been suggested as vectors for this organism, because catscratches may be absent in some cases. Abdominal pain is common and occasionally is severe. Abdominal ultrasonography shows microabscesses in the liver or spleen. Positive serologic results for *B. henselae* establish the diagnosis. Several antibiotic regimens have proved effective (188). Among HIV-infected patients, a syndrome of peliosis hepatis (bacillary angiomatosis) is caused by *B. henselae* infection. Serologic assays, indirect immunofluorescence, and PCR assays may assist in diagnosis (189); prolonged antibiotic regimens with erythromycin, doxycycline, or macrolides have proved effective in various series (190,191).

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Chapter 51

Hepatobiliary Manifestations of Human Immunodeficiency Virus

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Key Concepts

- Liver disease has assumed a far greater importance as a cause of morbidity and mortality in patients infected with human immunodeficiency virus (HIV) because of their increased life expectancy as a result of antiretroviral therapy (ART).
- Hepatobiliary disease in HIV-infected patients can be divided into two groups: Those with severe immunosuppression, who commonly have opportunistic infections, and those with suppressed HIV viral loads and minimal immunosuppression.
- Because of shared modes of transmission, coinfection with hepatitis B virus (HBV) and hepatitis C virus (HCV) is common. HCV-related liver disease is an important cause of morbidity and mortality. HIV accelerates the natural history of HCV infection. Combination therapy with pegylated interferon and ribavirin is effective in treating these patients.
- Hepatotoxicity associated with ART is a major cause of morbidity in HIV-infected patients. Steatosis and HIV-associated lipodystrophy are emerging as distinct entities causing liver disease in this group of patients.
- HIV infection was earlier considered a contraindication to liver transplantation. In recent years, liver transplantation is emerging as a cornerstone in the management of end-stage liver disease in HIV-infected patients.

Since its recognition more than two decades ago, the natural history of infection with human immunodeficiency virus (HIV) has undergone a vast change, from a disease associated with high mortality, to a chronic illness that may last for decades without significant morbidity. With the introduction of antiretroviral therapy (ART) in 1996, HIV-infected patients have experienced dramatically prolonged survival, resulting in patients falling sick from comorbidities unrelated to HIV. Liver disease has become one of the most important factors affecting survival, quality of life, and health care costs among HIV-infected patients. HIV-infected patients experience an array of hepatic manifestations (Table 51.1).

Given the shared epidemiologic risks, patients with HIV are commonly coinfected with hepatotropic viruses, and in particular coinfection with hepatitis C virus (HCV) which is now the leading cause of liver disease in HIV-infected patients in developed nations. Hepatotoxicity associated with ART also contributes significantly to liver disease in HIV-infected patients. In recent years, with the effective use of ART, opportunistic infections have assumed a lesser role in contributing to liver disease, although they are still an important cause of morbidity in developing countries. Steatosis and HIV-associated lipodystrophy are now being considered as distinct entities in this group of patients. Hepatic manifestations of HIV-infected patients without significant immunosuppression result from coinfection as with other hepatotropic viruses. The natural history, pathogenesis, and management of the hepatitis viruses in the presence of HIV infection are discussed in the subsequent text.

Table 51.1. Major Causes of Liver Injury in HIV-Infected Patients

DRUGS
- ART: NRTI, NNRTI, PI
- Antimicrobial agents:
  - Antituberculosis (INH, rifampin)
  - Macrolides (clarithromycin, azithromycin)
  - Antifungal (ketoconazole, itraconazole, fluconazole)
  - Antipneumocystis (TMP-SMX, pentamidine, dapsone)

INFECTIONS
- Viral (HAV, HBV, HCV, HDV, GBV-C, CMV, HSV, VZV, EBV)
- Mycobacterial (*Mycobacterium avium*, *Mycobacterium tuberculosis*, other mycobacteria) Fungal (cryptococcus, histoplasma, coccidioides, candida)
- Protozoan (pneumocystis, toxoplasma, microsporidia, cryptosporidium)

BILIARY TRACT INFECTIONS
- HIV cholangiopathy
- Acalculous cholecystitis

NEOPLASMS AND VASCULAR LESIONS
- Kaposi sarcoma
- Lymphoma
- Peliosis hepatis

STEATOSIS WITH LIPODYSTROPHY
- HCV/HIV coinfection
- Drug-associated (PI and NRTI)

ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; INH, isonicotonic acid hydrazide; TMP-SMX, trimethoprim-sulfamethoxazole; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; GBV-C, hepatitis G virus; CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus.

Hepatitis B Virus

Because of common epidemiologic risks of transmission including sexual and parenteral exposures, coinfection of hepatitis B virus (HBV) with HIV is common. Coinfection rates vary by risk factors, geographic region, and endemicity, being the highest among men who have sex with men (6% to 10%) in developing countries (1,2,3). As a consequence, up to 80% of HIV-infected patients have serologic markers of present or past HBV infection (hepatitis B surface antibody [anti-HBs] or hepatitis B core antibody [anti-HBc] positive) and 10% to 15% are chronic HBV carriers (hepatitis B surface antigen [HBsAg] positive) (2,3,4). Decreased response to HIV-ART and a higher risk of hepatic decompensation was observed in HBV/HIV-coinfected patients compared with those of HIV-monoinfected patients (5). Chronic liver disease because of viral hepatitis has emerged as one of the leading causes of mortality and morbidity in HIV-infected patients in the post-ART era (6).

Immune Dysfunction in Human Immunodeficiency Virus and Hepatitis B Virus Coinfection

Immune dysfunction related to HIV infection affects the natural history of HBV infection, reflecting the fact that the immune response plays a key role in viral clearance and the hepatic damage associated with HBV infection (7). Cytotoxic CD8+ lymphocytes recognize HBV antigens in the context of human leukocyte antigen (HLA) class I antigen exposed on the surface of infected hepatocytes and destroy them. During clearance of virally infected cells, CD8+ lymphocytes, with CD4+ help, mediate destruction of infected hepatocytes with resultant transaminitis and histologic damage (8). As the CD4+ lymphocytes are crucial for effective CD8+ cell-mediated immunity (CMI), depressed CD4+ cell number and function caused by HIV-mediated destruction will modify the natural history of HBV infection. This is illustrated by the finding that increased risk of chronic infection appears to be inversely correlated with the CD4+ cell count and is likely a result of the inability to promptly and vigorously respond to HBV antigen (9). The importance of the CD4+ cells in the control of HBV is also illustrated by the finding that improvement of HIV-infected patient's immune response with ART may induce spontaneous e antigen (hepatitis B e antigen [HbeAg]) to e antibody (hepatitis B e antibody [HbeAb]) seroconversion of their chronic HBV infection (9). On the other hand, ART-induced immune reconstitution may also be associated with acute hepatitis, presumably because of increased recognition and destruction of HBV-infected hepatocytes (7,10). Decreased anti-HBV immune surveillance resulting from HIV also results in heightened HBV replication, manifested by higher levels of HBV-DNA, higher rates of reactivation of HBV infection, increased HBeAg titer, and a lower rate of spontaneous HBeAg seroconversion (9,11). Despite higher serum HBV-DNA levels, hepatic necro-inflammation tends to be milder in HBV/HIV-coinfected individuals (12) that is in agreement with the postulated immune-mediated pathogenicity of HBV. However, the enhanced replication levels of HBV in HIV-coinfected patients may result paradoxically in the progression of liver fibrosis and increased mortality (11). Consistent with this observation, several clinical studies have shown that the risk of end-stage liver disease is significantly increased in HIV-infected patients with chronic hepatitis B, especially those with low CD4 cell counts (1,2,3,4,5). The diminished immune responses in HIV-infected individuals may enhance a persistent HBV coinfection through covalently closed circular DNA (cccDNA) that

replicates in the cytosol of infected hepatocytes (8). The

term "occult HBV infection" was recently coined to define the presence of HBV DNA in the serum or liver of patients without HBsAg (13). The results of previous studies of occult HBV infection in HIV-infected patients are controversial. Earlier studies have shown high rates of occult infections (14,15). However, recent studies have found very low level (<1%) of such occult HBV infections (16,17). Therefore, the prevalence and potential impact of occult hepatitis B infections are still unclear in the setting of HIV infection.

Treatment

The goals of HBV therapy in patients with HIV/HBV coinfection are suppression of HBV DNA, seroconversion from HBeAg to anti-HBe, normalization of transaminases, and improvement of liver histology. Guidelines for HIV-negative individuals recommend that treatment should be considered for patients with detectable serum HBV DNA above 10^5 copies/mL in HBeAg-positive patients (17a). A lower threshold may be appropriate for HBeAg-negative patients and those with decompensated cirrhosis (thresholds of 10^4 and 10^3 copies/mL, respectively) (17a). At this time, recommendations for the treatment of HBV in the setting of coinfection with HIV should be taken individually and on the basis of consideration of several aspects, including some related to the HIV infection (17a). Drugs approved for the treatment of chronic HBV infection are interferon (IFN), peginterferon α-2a, lamivudine, adefovir dipivoxil, and entecavir. Moreover, other compounds, such as tenofovir disoproxil fumarate and emtricitabine, approved for HIV therapy, also show strong anti-HBV activity. Although not approved for use against HBV, these drugs are often used in HIV-infected patients. Because HBV virions use a reverse transcription step in its life cycle, similar to HIV, nucleoside reverse transcriptase inhibitors (NRTIs) have been used to treat HBV-infected patients. The first NRTI to be used was zidovudine (formerly called *azidothymidine* [AZT]), but when administered in combination with IFN, AZT was no more efficacious than when administered with IFN alone (8). Greater success has been achieved with lamivudine, a potent inhibitor of the reverse transcriptase of HIV and HBV. U.S. Food and Drug Administration (FDA) has approved lamivudine for the treatment of chronic HBV infection (100 mg/day), as well as for the treatment of HBV infection as a component of an ART regimen (300 mg/day). However, the use of lamivudine is limited by the development of a high resistance rate because of a mutation in the (tyrosine, methionine, aspartate, aspartate) YMDD motif of the HBV-DNA polymerase gene (18). In patients with HBV infection alone, resistance ranges from 24% in the first year of treatment to 67% by year 4 (18); in HIV/HBV-coinfected patients, resistance is more frequent, with 47% resistant to lamivudine at year 2 and 90% at year 4 (19). Among patients with the YMDD mutation, many have a rebound in HBV viremia with a subsequent diminished clinical response to lamivudine therapy, aminotransferase flares, and occasional fatal liver failure (20,21). Emtricitabine, a close analog of lamivudine, has been approved for the treatment of HIV infection in the United States and is also active against HBV infection (22). Emtricitabine induces a rapid and sharp reduction in HBV-DNA levels (mean -3·4 $\log_{10}$ in 2 months) at doses of 25 to 300 mg/day (22). Suppression of HBV replication is maintained over 48 weeks of treatment in more than one-half of patients (22). However, not enough data are available on its use in HBV/HIV-coinfected patients. Famciclovir, an acyclic guanine derivative, suppresses HBV replication modestly, but in combination with

lamivudine, shows synergistic antiviral effect (23). Famciclovir-resistant HBV mutations different from lamivudine mutations have been documented during famciclovir monotherapy.

Adefovir dipivoxil is the bioavailable prodrug of adefovir, a nucleotide analog of adenosine monophosphate that was under development for HIV treatment until its use was halted because of nephrotoxicity at doses higher than 30 mg (24). As with lamivudine, the approved HBV infection treatment dose (10 mg/day) is lower than the HIV treatment dose (30 mg/day). Adefovir suppresses HBV DNA replication by 3.52 to 3.91 $\log_{10}$ copies/mL from baseline (25); normalizes transaminase levels in up to 70% of patients with HBeAg-negative chronic hepatitis (24); and causes HBeAg seroconversion in 23% of HBeAg-positive patients (24). Adefovir is also effective in lamivudine-resistant patients (26) and HIV-coinfected patients (27). The use of adefovir may lead to resistance too. The mutations, rtAsn236Thr and rtAla181Val, have been shown to be selected in 3·1% of HBV-monoinfected individuals treated with adefovir monotherapy after 3 years of follow-up (28,29). Tenofovir disoproxil fumarate is a nucleoside analog closely related to adefovir approved for the treatment of HIV. It is not yet approved for treatment of HBV, but reports demonstrate efficacy against both wild-type and lamivudine-resistant HBV strains, even in the presence of lamivudine-resistance mutations (30,31). Increased dosing intervals are also recommended for patients with abnormal renal function to avoid rare renal toxicity (32). A number of studies showed the potent effect of tenofovir in patients with HIV and HBV coinfection, particularly patients who had developed lamivudine resistance (31,33,34). A recent study by van Bommel et al. compared adefovir, 10 mg/day versus tenofovir, 300 mg/day in patients with lamivudine resistance (35). The tenofovir group, that included both HIV seronegative and coinfected patients, had a significantly earlier

response to treatment and a greater mean HBV DNA $\log_{10}$ drop (5.5 vs. 2.8 log) at week 48 than the adefovir group. In addition, all 35 of the patients treated with tenofovir achieved undetectable viral loads at week 48 compared with only 44% of patients taking adefovir. There was also a better serologic response in the group treated with tenofovir with 11 of the patients seroconverting to anti-HBe and 5 of these patients having HBsAg loss corresponding to a cure of their infection (35). There have not been reports of nephrotoxicity in patients on tenofovir therapy for HBV. In clinical studies assessing the efficacy of tenofovir against HBV in HBV/HIV-coinfected patients, HBV DNA levels declined on average 4 $\log_{10}$ with less than 25% of anti-HBe serocoversion, despite most patients carrying lamivudine-resistance mutations in both lamivudine-experienced and naïve patients (31,33,34,35,36,37). Tenofovir may be more potent than adefovir in controlling HBV in patients also infected with HIV (38). At 48 weeks, the mean change in HBV-DNA levels from baseline was -4·4 $\log_{10}$ in the group treated with tenofovir and -3·21 $\log_{10}$ in the group treated with adefovir (38). Nephrotoxicity mediated by renal tubular damage in HIV patients treated with ART has been reported (39). Entecavir, another nucleoside analog, was recently approved by the United States regulatory authorities for treatment of HBV infection (40). Entecavir is active against wild-type and lamivudine-resistant HBV in vitro and in vivo (41). The drug is specific for HBV and lacks any anti-HIV activity and it may be a better choice of therapy for HBV/HIV-coinfected patients who do not need HIV therapy because it does not promote evolution of HIV-resistant virus. Virologic responses to entecavir are seen in patients with lamivudine-resistant HBV, although they tend to be lower than in lamivudine-naive patients. Although

a number of resistance mutations were found in vitro, clinical resistance to entecavir requires multiple mutations in HBV polymerase in vivo, including those linked to lamivudine resistance (42). Therefore, entecavir doses of 0.5 mg/day are recommended in drug-naive patients, and doses of 1.0 mg/day daily are recommended for patients with lamivudine-resistant HBV strains (17a). A recent international trial with 68 HBV/HIV-coinfected patients with 88% lamivudine-related mutations demonstrated at least a 2 $\log_{10}$ copies/mL HBV viral load drop from baseline after 24 weeks of treatment (43).

Recently, the FDA approved peginterferon α-2a for the treatment of chronic hepatitis B infection in patients positive and negative for HBeAg with a higher HBeAg seroconversion rate than that of lamivudine (32% vs. 19%, respectively) (44,45). Peginterferon is more effective in HBeAg-positive patients than HbeAg-negative patients (32% vs. 19%, respectively). Although treatment of HBV with peginterferon has a reasonable success rate in immunocompetent patients, its value in HBV-infected patients with HIV is not known. As mentioned, immunosuppressed HIV-infected patients have higher levels of HBV DNA, lower pretreatment transaminases levels and less hepatic inflammation (4,5,12). These are all predictors of poor response to IFN treatment against HBV. Earlier studies showed that HIV-positive patients are approximately one-fifth as likely to respond to a 12-week course of IFN, clearing HBeAg in less than 10% of patients (46). This is especially true in patients with low CD4 cell counts. However, therapy with IFN may decrease the incidence of HBV cirrhosis regardless of HIV status (47). IFN is contraindicated in patients with decompensated cirrhosis because of the risk of serious bacterial infections and bone marrow suppression (17a). IFN appears to be safe in patients with compensated cirrhosis, although there is a risk of hepatic decompensation with prolonged therapy (17a).

New compounds are currently being tested for the treatment of HBV infection. Unlike the other analogs, Telbivudine (LdT) does not possess anti-HIV activity, it is expected to be approved by the FDA in 2006. Diaminopurine dioxolone (DAPD) and clevudine (L-FMAU) are nucleoside analogs in early clinical investigation that are active against wild-type and lamivudine-resistant HBV. Elvucitabine (β-L-FD4C), another L-deoxycytidine analog, is also a potent antiviral against HBV and HBV in animal models.

As HBV cure is a rare event, maintenance of therapy with long-term nucleoside/nucleotide analogs may be required in most cases. The role of combination therapy in the treatment of HBV is unclear at this time; however, emtricitabine (or lamivudine) plus tenofovir or emtricitabine plus adefovir may be good first choices in HBV/HIV-coinfected patients requiring ART (17a). For HBV/HIV-infected patients not requiring ART, entecavir or peginterferon may be reasonable options. In cases of no clear indication for anti-HBV treatment, the inclusion of active drug(s) against HBV in the ART regimen may be considered to avoid hepatic flares in the context of immune reconstitution (17a).

Hepatitis D Virus

Hepatitis D virus (HDV, delta agent) is a defective RNA virus that requires concurrent infection with HBV (coinfection) or chronic HBV carriage (superinfection) to produce hepatic disease. HDV superinfection causes a more severe liver disease than HBV infection alone, with progression to cirrhosis in 70% to 80% of immunocompetent patients (48). The incidence of this virus is low in the homosexual population, but up to 70% of parenteral drug users carry HDV, many of whom are also HIV-positive (49). Evolution towards cirrhosis tends to be

faster, and the outcome is generally much worse in these multiple coinfected patients

than in individuals with monoinfections (48). HDV is directly cytopathic to hepatocytes, and disease appears to be worsened by immunosuppression, although some observational studies have failed to show an influence of HIV on the clinical course of HDV infection (49). This may be because of the small number of patients studied. HIV infection does not appear to alter the efficacy of IFN treatment for HDV infection; however, the long-term response is poor in both groups even with lamivudine (50). Finally, coinfection with HCV rarely responds to combination therapy with IFN plus ribavirin when delta virus is present (49,50).

Hepatitis A, E, and G Virus

The prevalence, morbidity, and mortality of Hepatitis A virus (HAV) infection are not altered by HIV infection. Despite this, vaccination of HIV-infected patients is recommended, particularly for homosexual patients who are at a higher risk of transmission through person-to-person sexual contact. The presence of chronic HCV appears to increase the risk for fulminant liver disease caused by acute HAV infection, so coinfected patients are excellent candidates for HAV vaccination (51). Hepatitis E virus (HEV), a waterborne or fecal orally transmitted virus, usually causes an acute self-limited disease. A study showed an increased incidence of HEV in HIV-infected patients from a nonendemic area, but without clinical significance (52). A putative hepatotropic virus, hepatitis G virus (GBV-C), is present in approximately 20% to 40% of the HIV-infected patients compared with less than 3% in the general population (53). The presence of GBV-C also does not alter CD4+ cell count or plasma HIV-1 RNA levels in coinfected patients. Although GBV-C does not cause clinical disease, HIV replication is inhibited by GBV-C, translating clinically into an increased survival of HIV-infected patients also infected with GBV-C (54). This virus seems to be a lymphotropic virus that may downregulate cytokines responsible for HIV replication. Another putative hepatotropic virus, transfusion-transmitted virus (TTV) is highly prevalent and replicates to a greater degree in HIV-infected patients, although the significance remains unknown (55). The prevalence of SEN virus and its implications among HIV-infected individuals remain unstudied.

Hepatitis C Virus

In the United States and other developed nations at least 2% of the population is infected with HCV (56). Among all HIV-infected patients in the United States, approximately 16% are coinfected with HCV (57). In addition to both being major health issues, there are many similarities between HIV and HCV infection. Both viruses possess a single strand RNA genome and result in subclinical chronic infection. Each virus is able to evade the host's immune system because of high genetic variability, and the replication rate of both viruses is extremely high. With the introduction of ART, there has been a dramatic improvement in survival of HIV-infected patients (58,59,60). In one year HIV virus can be suppressed to undetectable levels in 70% to 80% of patients. With longer survival patients with HIV infection/disease now increasingly fall sick from comorbid diseases, chief among them being HCV infection. HCV is not classified as an opportunistic infection in HIV-infected patients, but as HIV can accelerate HCV-induced liver disease, it may actually mimic an opportunistic infection (61). Chronic HCV increases both morbidity and mortality in HIV-infected patients. Although overall

mortality of HIV-infected patients has declined, the relative mortality because of liver disease has increased. A 5-year study of a cohort of HIV patients in the ART era found an increased mortality in HIV/HCV-coinfected patients versus HIV-monoinfected patients, with a mortality of 6.7 versus 2.3/100 person years (P = 0.05). Hospitalization of the coinfected group was also more common compared with the HIV-monoinfected group (62). A large French cohort study of more than 17,000 HIV-infected patients compared mortality in pre-ART era to ART era. Although there was a decline in mortality from all causes from 2% to 0.9%, an increase in deaths related to cirrhosis and hepatocellular carcinoma (HCC) from 6.5% to 10% was observed. Coinfection of HCV-infected patients with HIV is common because of shared modes of transmission. The prevalence HCV coinfection varies according to the risk for HIV acquisition; amongst intravenous drug users up to 95% are HCV positive (57,63) and amongst hemophiliacs 73% are HCV positive (57). On the other hand in those belonging to low-risk categories for HCV infection including men who have sex with men, heterosexual persons with exposure to HIV, and health care workers, the prevalence of coinfection is much lower at about 3.5% (57). Vertical transmission of HCV may be increased up to threefold in presence of HIV.

Impact of Human Immunodeficiency Virus and Antiretroviral Therapy on Hepatitis C Virus

In addition to influencing the transmission rate of HCV, HIV also significantly affects the natural history of HCV-related liver disease. In comparison with HBV infection, hepatic damage resulting from HCV infection

is predominantly caused by direct viral toxicity with lesser contribution from the host immune response. Cell-mediated immunity, particularly Th 1 clones play a role in viral clearance through recognition of multiple core epitopes of HCV on virally infected hepatocytes (64,65,66). Given this role, the decline in CD4+ cells associated with progressive HIV infection permits greater HCV replication. In addition to suppressing an active cell-mediated response to HCV, coinfection with HIV also appears to alter the quality of that response. When $CD3^+/CD30^+$ cells are infected with both HCV and HIV, their cytokine production is skewed towards an anti-inflammatory Th 2 response rather than the protective Th 1 response seen when cells are infected with HCV alone (67). Viral escape from cytotoxic CD8+ lymphocytes may also result in enhanced viral replication. Similar to HIV, viral evolution is seen through accumulation of mutations resulting from the high replication rate of this virus in the face of selective pressure from lymphocytes directed against HCV antigens. The HCV hypervariable region, which is a major target of cell-mediated immunity and humoral mechanisms, exhibits greater heterogeneity in HIV-infected patients (68). HCV viral loads are higher in plasma (69,70) and liver (71) in HIV/HCV-coinfected patients compared with HCV-monoinfected patients because of greater HCV replication reflecting the immunosuppressed state. There is a more rapid progression to cirrhosis, end-stage liver disease and HCC in coinfected patients (72,73,74,75,76). Other factors associated with more rapid HCV-related fibrosis are low CD4+ counts, alcohol use, and a higher age at initial HCV infection. However, many of these studies confirming a more rapid progression of liver disease in HIV/HCV-coinfected patients were carried out in the pre-ART era. A number of studies have attempted to assess whether ART affects HCV replication and progression. Although ART causes hepatotoxicity, it is also possible that ART-induced immune restoration might attenuate the increased risk of cirrhosis associated with

coinfection. Earlier studies suggest that protease inhibitors (PIs) did not inhibit HCV replication (77), and the lowering of HIV viral load by ART did not affect HCV viral load (78). One study (79) has shown that ART may retard the progression to fibrosis. In 182 coinfected patients the progression to fibrosis was slower in those on ART, which included a PI, as compared with those who did not receive a PI. The estimated 15-year rate of progression to cirrhosis was 5% versus 18% ($P < 0.001$). In another report it was seen that use of PI-based ART was associated with lower risk of bridging fibrosis or cirrhosis, whereas nevirapine (NVP) was associated with greater risk of disease (80). In a recent study addressing the role of ART in coinfected patients, out of 210 coinfected patients, 64% had received ART within 2 years of liver disease assessment. Of these, 33% had no fibrosis and 23% had bridging fibrosis or cirrhosis. Significantly lower hepatic necroinflammatory scores (mean-3, range 0 to 9 of 18) were observed among persons who received ART longer ($P = 0.02$) and who had HIV RNA suppression ($P < 0.01$). This study suggests that ART use was not protective against fibrosis, although individuals with longer cumulative exposure to ART and HIV RNA suppression had significantly less necroinfammatory activity (81). Identification of noninvasive markers of fibrosis (liver enzymes, albumin, and hyaluronic acid) may help in identifying individuals at greatest risk for cirrhosis (82). Some degree of steatosis has been observed in up to 40% of patients with HCV/HIV coinfection. Patients with steatosis are more likely to have greater hepatic fibrosis and more severe HCV-related liver disease. Obesity, hyperglycemia, and use of stavudine (d4T) have been identified to be modifiable risk factors for steatosis in this population (83). HCC in HIV has a more aggressive course with a higher frequency of infiltrating tumors and extranodal metastases at presentation, portal invasion and reduced survival (84,85). Progression from initial HCV infection to HCC is more rapid in coinfected patients, probably because of a more rapid progression to cirrhosis. Despite a more rapid rate of liver disease progression, the impact of coinfection with HIV on mortality is unclear. Although some studies report a higher liver related mortality (86), others have not shown any effect on survival (87,88). In the ART era, longer follow-up will be necessary to show a difference in mortality in the two groups.

Impact of Hepatitis C Virus on Human Immunodeficiency Virus

HCV may act as a cofactor for HIV disease progression by several mechanisms. The state of permanent immune activation provided by chronic HCV infection can favor HIV transcription within infected cells and cause more rapid destruction of CD4 T lymphocytes (68). Moreover the immune response to ART may be slowed down in the presence of HCV infection (89). Data regarding the influence of HCV on the natural history of HIV diseases is conflicting. The Swiss cohort study with 1,157 patients demonstrated that the risk of HIV progression to clinical acquired immunodeficiency syndrome (AIDS) or death was independently associated with HCV seropositivity (90). On the other hand, in an urban United States cohort (91) of 1,955 patients, HCV infection did not substantially alter the risk of death, clinical AIDS, or the response to ART. Various other studies have also shown mixed results (92,93,94). HCV may also negatively influence HIV disease by resulting in increased liver toxicity and higher treatment discontinuation (95,96).

Diagnosis

All patients with HIV should be screened for HCV. The method of screening is

important as the enzyme-linked immunosorbent assay-2 antibody test (anti-HCV ELISA-2) can result in an estimated 3% to 4% false-negative rate (57,97) in coinfected patients because of impaired HCV antibody production with immunosuppression, a rapid decline in antibody titer, and a possible interaction between the two viruses. Screening with HCV RNA should be done in coinfected patients by either the more sensitive qualitative tests or by quantitative methods using target amplification or signal amplification techniques. The role of liver biopsy in treatment decision has been controversial in HIV/HCV-coinfected patients. Given the effect of HCV on morbidity and mortality, the complexities of therapy for both HIV and HCV infection, and the current limitations of noninvasive markers, liver biopsy continues to play an important role in the evaluation of HCV infection in the setting of HIV coinfection.

Management

Given the impact of HIV-induced immunosuppression on natural history of HCV disease, infection with HCV should be treated as any other opportunistic infection. The goals of treatment of HCV in the presence of HIV are viral eradication, slowing the progression of liver disease, and better tolerance of anti-HIV medication. The adverse effects of anti-HCV therapy and drug interactions must be weighed with the benefits of treatment. The various treatment options and evidence supporting their use are discussed in the subsequent text.

Interferon monotherapy: IFN inhibits virus-induced cytopathic effects, induces natural killer cells and cytotoxic T lymphocytes, and has a role in chemo-attraction of these cell lines to the liver. It stimulates major histocompatibility complex (MHC) Class 1 expression and acts to polarize the adaptive immune response to a Th 1 milieu. In HIV-coinfected patients the end of treatment response to IFN monotherapy has been found to be similar to HCV-monoinfected patients, but sustained virologic responses (SVRs) are rare (98,99). Histologic response has also been observed with IFN monotherapy. Di Martino et al. (100) compared the histologic response to IFN in patients with coinfection and those with HCV alone in a cohort of 79 patients. The rate of histologic improvement (2 point drop in Knodell score on paired liver biopsies) was similar in the two cohorts, but the SVR was much lower in the coinfected group (6% vs. 30%). Treatment with IFN did not significantly change CD4 counts or the HIV RNA levels.

Interferon and ribavirin: Ribavirin is a guanidine analog that has an immunomodulatory action; it increases the production of Th 1 cytokines and decreases the production of Th 2 cytokines. Combination therapy with standard IFN and ribavirin in naive monoinfected HCV patients achieves an SVR of 17% to 29% in patients with genotype 1 and 65% to 67% in genotype 2 and 3. In HIV/HCV-coinfected patients, combination therapy appears to be well tolerated and SVR rates are better than those with IFN alone. The American Foundation for AIDS Research Study DCRI 010 (101), in a randomized controlled trial in HIV/HCV coinfection used therapy with IFN-2b at 3 MIU three times weekly combined with either initial or 16-week delayed ribavirin at 800 mg/day in 106 patients. The response at week 12 was 23% in the combination group compared with 5% in IFN and placebo group (P = 0.016), proving that even in coinfection the addition of ribavirin improves initial response. However, the difference in SVR between the two groups was not statistically different (11% vs. 5%). In the Hepatitis Resource Network study HRN-002, a controlled trial of 155 coinfected

patients showed that daily IFN in combination with ribavirin was more effective in obtaining an SVR than a standard IFN regimen given thrice weekly (19% vs. 8.6%, *P* < 0.001). In this study, early virologic response (EVR) was a strong predictor of SVR (102).

Pegylated interferon and ribavirin: The use of standard IFN has now been largely replaced by peginterferon, and treatment with peginterferon in combination with ribavirin is now the standard of care in managing HIV/HCV-coinfected patients. In the largest study so far, the AIDS Pegasys Ribavirin International Coinfection Trial (APRICOT) (103), a total of 868 subjects with HCV and HIV coinfection were assigned to receive one of three regimens: Peginterferon α-2a (180 μg/week) plus ribavirin (800 mg/day); peginterferon α-2a plus placebo; or IFN-α-2a (3 million IU thrice a week) plus ribavirin. Baseline characteristics were well-matched between the three groups. The overall rate of SVR was significantly higher in the group receiving peginterferon and ribavirin than in that receiving standard IFN and ribavirin (40% vs. 12%, *P* < 0.001), or peginterferon plus placebo (40% vs. 20%, *P* < 0.001). SVR in genotype 1 was 29% in group 1, which is higher than the previous studies. Genotypes 2 and 3 were treated for 48 weeks and achieved a SVR of 62% in the group treated with peginterferon and ribavirin. In the group treated with peginterferon and ribavirin, a baseline HCV viral load of more than 800,000 IU/mL led to a significantly lower

SVR rate in genotype 1 as compared with those with viral load less than 800,000 IU/mL (18% vs. 61%), but not in genotypes 2 and 3 (63% vs. 61%). Multivariate logistic regression analysis identified genotype non-1 and HCV RNA level less than 800,000 IU/mL, as independently associated with higher SVR. Age, race, liver histology, CD4+ counts, and ART did not influence SVR rates. In the peginterferon and ribavirin group, the HIV viral load decreased by 0.7 log 10, which supports the concept that IFN leads to decline in HIV viremia. In the AIDS Clinical Trials Group (ACTG) study (104), a total of 66 patients were randomly assigned to receive 180 μg of peginterferon α-2a weekly for 48 weeks along with ribavirin escalating from 600 to 1,000 mg/day. Sixty-seven patients were given 6 million IU of standard IFN thrice a week for 12 weeks followed by, 3 million IU three times a week for 36 weeks along with the same dose of ribavirin. At the primary end point, week 24, viral response was better in the peginterferon rather than the IFN group (44% vs. 15%; *P* < 0.001). Treatment with peginterferon and ribavirin was associated with a significantly higher rate of SVR than treatment with standard IFN and ribavirin (27% vs. 12%, *P* = 0.03). In the group given peginterferon and ribavirin only 14% of patients with HCV genotype 1 had a SVR, as compared with 73% in other genotypes. Independent factors associated with higher SVR were genotype non-1 infection, no prior intravenous drug use, and an undetectable HIV RNA at baseline.

Although a slower decline in HCV RNA level is seen in coinfected patients after standard therapy is initiated with peginterferon and ribavirin, the stopping rule at week 12 that is recommended for HCV-monoinfected patients seems to be equally valid in HIV-positive patients (103,104,105). In the ACTG trial (104), of the 106 subjects in whom HCV RNA levels were measured at week 12, 43 (41%) had an EVR. Twenty-two of them (51%) had a SVR; in contrast none of the 63 patients who did not have an EVR achieved a SVR (negative predictive value 100%). Similarly, in the APRICOT

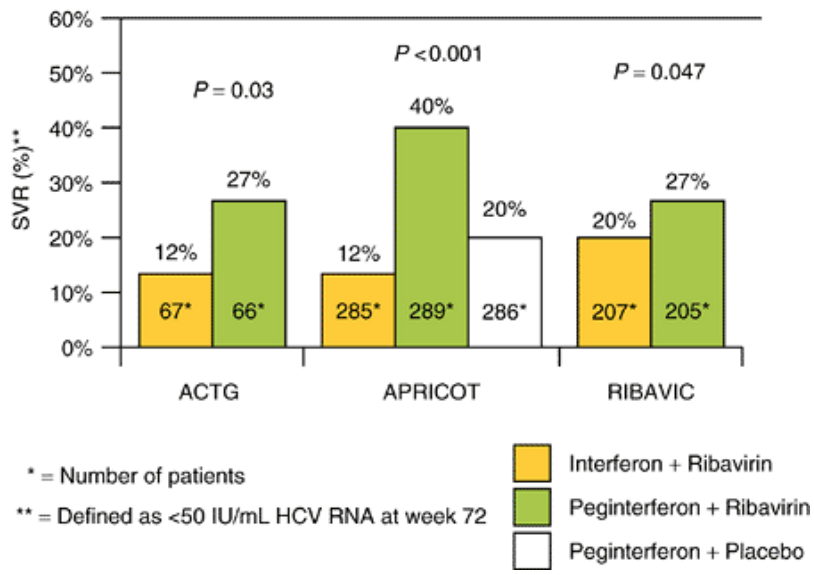
(103) study only 2% of the patients who did not have an early response went on to have an SVR (negative predictive value 98%). When an EVR was defined as one occurring at week 24, the results were similar. In a large French randomized controlled trial of 412 HIV/HCV-coinfected patients, the RIBAVIC study (105), 48 weeks of weekly peginterferon in combination with 800 mg/day ribavirin was compared with thrice a week standard IFN and ribavirin treatment. SVR was achieved in 20% of IFN patients versus 27% of peginterferon patients (P = 0.047). However, the peginterferon therapy was superior to standard IFN therapy only in genotype 1 or 4 (17% vs. 6%; P = 0.01), but not in genotypes 2 and 3 (44% vs. 43%; P = 0.88). Similar to the APRICOT and ACTG studies, the week 12 EVR had a high negative predictive value of 99%. In a smaller randomized controlled trial (106), 95 patients were randomly assigned to receive either peginterferon at 100 µg (<75 kg) to 150 µg (>75 kg) once weekly or standard IFN 3 million IU thrice a week, each combined with ribavirin 1,200 mg/day (>75 kg), 1,000 mg/day (60 to 74.9 kg), and 800 mg/day (<60 kg). Treatment was given for 48 weeks in genotype 1 and 4, and for 24 weeks in genotype 2 or 3. The peginterferon plus ribavirin group had a higher SVR in genotypes 1 and 4 as compared with the IFN plus ribavirin group (38% vs. 7%; P = 0.007). However, in genotypes 2 and 3 there was no significant difference in SVR between the two groups (53% vs. 47%; P = 0.73). This and the RIBAVIC (105) studies suggest that the peginterferon and ribavirin treatment has superior SVR rates only in genotype 1, and not in genotypes 2 and 3. This study used a higher dose of ribavirin and showed SVR of 38% in genotype 1. This is higher than those reported in other studies, suggesting that HIV/HCV-coinfected patients may have a higher SVR with a higher dose weight–based ribavirin regime. In summary, therapy with peginterferon and ribavirin is superior to standard IFN and ribavirin, and achieves overall SVR rates of up to 40% (Fig. 51.1). The difference is more marked with genotype 1 as compared with genotypes 2 and 3.

Adverse Effects of Therapy

Common side effects of IFN and ribavirin therapy include fatigue, influenza-like symptoms, hematologic abnormalities, gastrointestinal disturbances, and neuropsychiatric symptoms. Both standard interferon and peginterferon have similar side effect profiles and discontinuation rates. In the APRICOT (103) study the rate of premature discontinuation was 12% in the peginterferon plus ribavirin group. The dose of peginterferon was reduced in 34% because of neutropenia and thrombocytopenia. The dose of ribavirin was reduced in 16% because of anemia. Similarly, in the ACTG (104) trial the treatment discontinuation rate was 12%, whereas in the RIBAVIC (105) study the discontinuation rate was 17% and serious side effects were seen in 32% cases. There are specific toxicities observed in this population as a result of having a concomitant chronic illness, drug

interactions, and higher susceptibility to the usual side effects of antiviral therapy. A clinically significant interaction is seen between IFN and AZT, leading to anemia from bone marrow suppression. The more profound anemia seen during HCV therapy in coinfected patients is a combined effect of hemolytic anemia caused by ribavirin and the failure of the suppressed marrow to compensate for the loss of red blood cells. Anemia in this setting can be managed by reducing

the dose of ribavirin and avoiding AZT wherever possible. Erythropoetin is effective in treating anemia in coinfected patients undergoing anti-HCV therapy and has an efficacy similar to monoinfected patients (107,108). Recent studies have not found a difference in neutropenia in patients receiving IFN plus ribavirin with or without concomitant AZT (101,103). Moreover, neutropenia does not lead to a higher rate of infection in this group of immunosuppressed patients (102). Ribavirin enhances the phosphorylation of didanosine (ddI) resulting in an increased risk of mitochondrial toxicity, pancreatitis, lactic acidosis, and fulminant hepatic failure (109). As a result the FDA has issued a black box warning for the use of ribavirin and ddI. Toxicity may also be seen with other NRTI particularly those with a high affinity for the mitochondrial enzyme DNA polymerase γ. d4T, zalcitabine (ddC), and ddI should be avoided. A reasonable approach would be to avoid combining these drugs with ribavirin and to use a less hepatotoxic agent like lamivudine, tenofovir, or abacavir. Liver enzymes, amylase and lactate should be monitored monthly while using the drugs. Liver decompensation during the treatment is also a complication of anti-HCV therapy in the coinfected patients. The rate of hepatic decompensation in this group of patients with cirrhosis is 7.8% to 10.4% (103,105). In the APRICOT study four factors were found to be associated with decompensation: Increase in total bilirubin, increase in alkaline phosphatase (ALP), fall in hemoglobin and use of ddI for HIV therapy. Therefore, coinfected patients with cirrhosis should be closely monitored during anti-HCV treatment and ddI should be avoided wherever possible. Active psychiatric illness, and active alcohol and drug use, are other barriers to anti-HCV treatment in coinfected patients (110). Current treatment with peginterferon and ribavirin is generally well tolerated with adverse events similar to those observed in HCV-monoinfected patients. Benefits of the treatment must be weighed against the risks of therapy, and the treatment should be individualized in this group of patients. Judicious use of erythropoietin, granulocyte colony stimulating factor (GCSF), and antidepressants can improve tolerance to treatment and outcome of therapy.



▪ **Figure 51.1** Overall sustained virologic response rates (SVR) of pegylated interferon and ribavirin in HIV/HCV coinfection in various clinical studies. HIV, human immunodeficiency virus; HCV, hepatitis C virus; ACTG, AIDS

Clinical Trials Group; APRICOT, AIDS Pegasys Ribavirin International Coinfection Trial.

Approach to Management of Human Immunodeficiency Virus/Hepatitis C Virus Coinfection

The 2002 National Institutes of Health (NIH) Consensus Development Conference on the management of HCV recommends treatment of HIV/HCV coinfection on a case-by-case basis (56). Several factors should be considered when deciding to start anti-HCV or anti-HIV therapy in coinfected patients (Fig. 51.2).

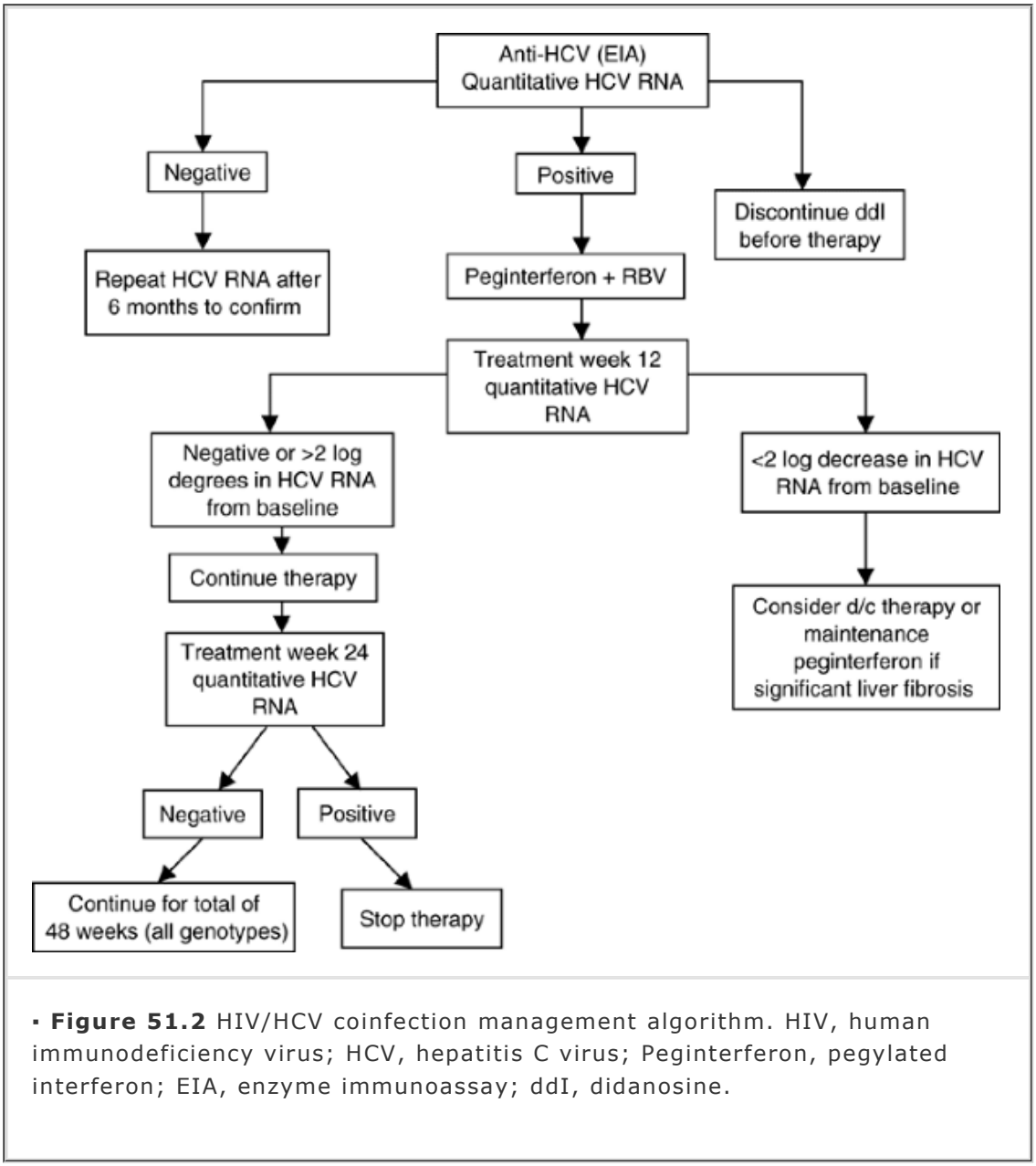
P.1410

HIV therapy with ART may slow the progression of HCV-induced liver fibrosis, moreover, immune reconstitution associated with PIs can help reduce the HCV viral load, and therefore, initiating ART therapy may improve the efficacy of anti-HCV therapy and should be a priority in managing the coinfected patient. On the other hand, chronic HCV infection is an independent risk factor for ART-induced hepatotoxicity. It has been shown that prior treatment of HCV infection decreases the rate of severe hepatotoxicity with ART. In a study evaluating 66 patients pretreated with anti-HCV therapy, and 39 patients without HCV treatment, the rate of discontinuation of ART was significantly higher in the untreated group as compared with those who were previously treated for HCV ($P < 0.01$) (111). Anti-HCV therapy should be initiated in all patients with elevated transaminases, stable HIV disease (HIV RNA <50,000), high CD4 counts (>350 cells/μL), and no opportunistic infections. Favorable HCV genotypes (2 and 3), advanced stage of fibrosis, and compensated liver disease also influence the decision to start anti-HCV treatment. Individuals with active alcohol and substance abuse, and prior severe neuropsychiatric illness should not be treated. In patients with low CD4 counts (<200), high HIV RNA levels and presence of opportunistic infections, HIV treatment takes priority. Treatment in patients with normal transaminases should be on the basis of the presence of significant liver fibrosis on biopsy. Patients with CD4 counts between 200 and 350 cells/μL, should be treated with caution (112). In contrast with HCV-monoinfected patients, HIV/HCV-coinfected patients with all genotypes should be treated with peginterferon and ribavirin for a period of 1 year. Patients with advanced fibrosis and cirrhosis should be treated for a year in order to delay the clinical and histologic progression of diseases. Maintenance therapy with peginterferon may also be considered in such patients even if HCV clearance is not achieved. In the recent ACTG trial (104) it was found that in subjects without a virologic response at week 24 who underwent liver biopsies, over one third had histologic evidence of improvement,

P.1411

suggesting a role for maintenance therapy. Patients with decompensated liver disease should not be treated with anti-HCV therapy, given the high risk for treatment-related side effects. Management in these patients is focused on symptomatic treatment, and referral for liver transplantation. In a retrospective cohort study (113), survival of 1,037 HCV-monoinfected and 180 HCV/HIV-coinfected patients with cirrhosis after first hepatic decompensation was analyzed. Out of these, 386 (37%) HCV-monoinfected patients and 100 (56%) coinfected patients died during follow-up. The median survival time of HIV-infected and HIV-noninfected patients was 16 and 48 months respectively ($P <$

0.01). HIV coinfection reduces the survival of patients with HCV-related end-stage liver disease, and this factor should be taken into account for proper timing of liver transplantation in these patients.



▪ **Figure 51.2** HIV/HCV coinfection management algorithm. HIV, human immunodeficiency virus; HCV, hepatitis C virus; Peginterferon, pegylated interferon; EIA, enzyme immunoassay; ddI, didanosine.

Hepatotoxicity Associated with Antiretroviral Therapy

Drug toxicity secondary to antiretroviral (ARV) and other agents as well as their interactions is a major concern in the treatment of HIV seropositive patients. Although asymptomatic and reversible in most cases, ART has been reported to cause severe (i.e., grade 3 or 4 inflammation; alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >5–10, and >10 times the upper limit of normal [ULN], respectively) liver injury requiring cessation of therapy in up to 30% of patients (114,115,116,117,118). ARV drug-specific factors such as hepatic metabolism, intrinsic effects, and interaction with the other drugs as well as patient-related factors including age, preexisting liver disease like chronic viral hepatitis, alcohol abuse, concomitant use of potentially hepatotoxic drugs,

and opportunistic infections may all contribute to the risk of liver toxicity. There are different mechanisms through which ARVs exert their toxic effects in the liver.

Hepatotoxicity Associated with Nucleoside Reverse Transcriptase Inhibitors

NRTIs are competitive inhibitors of viral reverse transcriptase terminating chain elongation of viral DNA. They have been associated with grade 3 or 4 hepatotoxicity in 5% to 6% of the patients in several cohort studies (115,119). However, very rarely they lead to steatosis, lactic acidosis, and liver failure with varying mortality rates (120,121,122,123). The underlying mechanism of liver injury attributed to NRTIs is associated with mitochondrial toxicity because of their inhibition of mitochondrial DNA polymerase-γ (124). This leads to impaired oxidative phosphorylation and fatty acid oxidation, which in turn results in hyperlactatemia and microvesicular steatosis. The degree of hepatotoxicity seems to be correlated with their potential to cause mitochondrial damage. Therefore, ddC, d4T, and ddI are the most common drugs in this group causing severe liver injury with life-threatening lactic acidosis in approximately 1% of the subjects (121,122,123,12). However, most patients have asymptomatic chronic hyperlactatemia. Hepatotoxicity typically occurs sometime after 3 months. Apart from the liver injury, mitochondrial toxicity is also manifested clinically as myopathy, neuropathy, and pancreatitis (126). Abacavir differs from the rest of the group in that although much safer in regard to lactic acidosis and subsequent liver injury, it is associated with a life-threatening hypersensitivity reaction manifesting within the first 2 weeks of initiation, which may also include liver dysfunction (126,127,128).

Hepatotoxicity Associated with Non-Nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) also inhibit HIV reverse transcriptase without nucleotide chain incorporation. Some of the previous studies raised concern that serious hepatic events are more common in patients treated with NNRTI-based (NVP in particular) regimens than NRTI or PI-based regimens (115,129,130). However, a recent analysis of hepatotoxic events from four cohorts (Amsterdam, CHORUS, ICONA, and TARGET) including 5,133 patients revealed that there was no consistent risk of long-term liver toxicity attributable to a specific ARV drug (131). In addition, another recent comprehensive review of 17 clinical trials and cohort studies between 1991 and 2001 derived from the FDA database demonstrated that the risks of developing grade 3 or 4 change with NVP or efavirenz-based therapies were similar to those associated with other ARV drugs (132). Similarly, serious clinical (symptomatic) hepatotoxicity was detected in 5% of those treated with NVP. NVP rarely causes a hypersensitivity reaction characterized by rash, eosinophilia, and fulminant hepatitis within the first 6 weeks of treatment that may be fatal (133,134). This type of reaction has been identified more often in women with relatively higher CD4 counts. Therefore, a warning has been issued by the manufacturers notifying the risk of hepatotoxicity, which is approximately 12-fold higher in women with CD4 count of more than 250 cells/mm^3 or men with CD4 counts of more than 400/mm^3. NVP should not be taken as HIV prophylaxis after needle stick exposure. The data also showed an increased risk of severe hepatotoxicity with NNRTIs in patients with concomitant chronic hepatitis B or C as well as in patients with elevated baseline transaminases

greater than 2.5 times the ULN (131,132,135). Overall, NNRTIs continue to be used because of their greater efficacy and tolerability.

Hepatotoxicity Associated with Protease Inhibitors

PIs interfere with the formation of viral reverse transcriptase. Some clinical studies have indicated that among all PIs, the use of full-dose ritonavir is associated with a higher risk of severe hepatotoxicity compared with other PI-based regimens and is the single most important predictor of grade 3 or 4 liver injury (115,118,136). However, recent data suggest that ritonavir associated liver toxicity is comparable to other PI-based regimens at lower doses to boost the levels of other PIs, like lopinavir or indinavir (137,138,139). Similar to NNRTIs, chronic hepatitis B or hepatitis C coinfection is associated with an increased risk of severe hepatotoxicity, particularly in patients not receiving full-dose ritonavir (115,116,118,129,136,140,141). On the other hand, most HCV-coinfected patients do not develop severe hepatotoxicity (139). Immune reconstitution that is an increase in CD4 count as a result of ART and subsequent acute hepatotoxicity with viral seroconversion has been suggested as a mechanism of PI-associated liver injury (142,143). But, this theory fails to explain ART-associated hepatotoxicity observed in subjects without viral hepatitis.

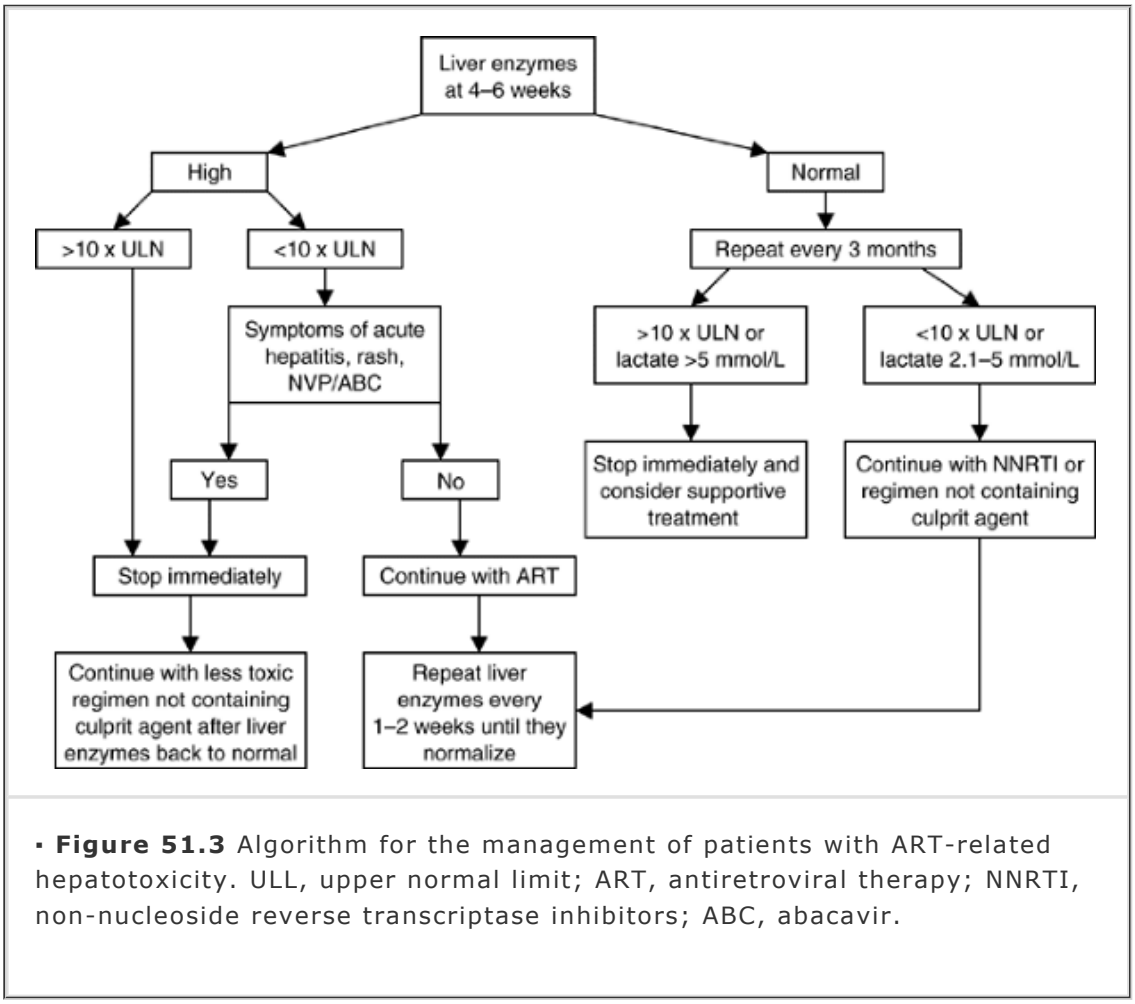
Clinically, the use of PIs may be associated with lipoatrophy, truncal-abdominal fat deposition, hyperlipidemia, insulin resistance (IR), and subsequent fatty liver (144). Indinavir may cause a hypersensitivity reaction whereas saquinavir and nelfinavir are more likely to act as direct hepatotoxins. In addition, indinavir and atazanavir are associated with asymptomatic indirect hyperbilirubinemia similar to Gilbert's disease as a result of their inhibition of uridine diphosphate (UDP)-glucuronosyl transferase activity.

Evaluation and Management of Patients with Hepatotoxicity

The most useful and analytic approach to a patient with drug-related toxicity is suggested by Nierenberg (145) addressing five specific questions: (i) Has this type of adverse reaction been observed previously? (ii) Was the timing appropriate? (iii) Does the problem improve with discontinuation of therapy with the drug? (iv) Does the problem recur with reexposure to the potential culprit? (v) Were the other likely causes of hepatitis excluded? Therefore, the possibility of ARV hepatotoxicity in a given patient should be assessed initially using this algorithm. Patients undergoing ART should have baseline liver function tests, screening for hepatitis A, B, C and also precise recording of alcohol consumption. Liver enzymes should be repeated at 4 to 6 weeks following initiation of ART and at least every 3 months thereafter if the values are within normal limits. Therapy must be completely withheld in case of liver enzyme elevation (LEE) within the first 4 to 6 weeks in patients undergoing treatment with NVP or abacavir because of the possibility of hypersensitivity reaction. Beyond 3 or more months, LEEs accompanied by nausea, vomiting, abdominal pain, fatigue, weight loss, and serum lactate levels between 2.1 to 5 mmol/L should prompt the physician to stop NRTI therapy or to replace it with a non-NRTI agent. In the absence of symptoms, serum lactate level greater than 5 mmol/L mandates withdrawal of all ART immediately, and to consider supportive treatment with vitamin B_1 (thiamine), B_2 (riboflavin), carnitine, coenzyme Q10, and/or vitamin C. In

patients without symptoms of acute liver failure or hyperlactatemia, the presence of LEEs less than or equal to 10 times the ULN requires repeating liver enzymes every 1 to 2 weeks until they improve or normalize as ART continues. However, LEEs exceeding 10 times the ULN, even in the absence of symptoms evoking liver failure warrant prompt withdrawal of ART. ART-induced hepatotoxicity is more likely if the regimen contains d4T, ddI, AZT, NVP or PIs, and no other cause of injury such as alcohol intake, concomitant drug toxicity, or hepatitis with other hepatotropic viruses (e.g., HAV, cytomegalovirus [CMV], herpes simplex virus [HSV] or Epstein-Barr virus [EBV]) is detected. A new regimen containing less hepatotoxic drugs like abacavir, lamivudine, or tenofovir may be considered after normalization of liver enzymes. An algorithm summarizing the approach to a patient with ART-associated hepatitis is depicted in Figure 51.3.

There are a variety of medications contributing to liver toxicity when taken together with ART. Among them, hydroxyurea in combination with NRTIs and particularly d4T/ddI, may cause acute liver failure with hepatic necrosis. As all PIs are metabolized in the liver by cytochrome P-450 (CYP450) system, concomitant medications that are also metabolized by CYP450 (e.g., isotonicotonic acid hydrazide [INH], rifampin, macrolides, ketoconazole) and PIs may potentiate hepatotoxicity of each other. In addition, caution is recommended when using statins in the treatment of PI-related hyperlipidemia as they may also contribute to hepatotoxicity. And finally, ddI and d4T, which have the greatest risk of mitochondrial toxicity, should not be coadministered with ribavirin in HIV/HCV-coinfected patients as ribavirin raises the intracellular levels of these agents increasing the frequency of hepatotoxicity.



▪ **Figure 51.3** Algorithm for the management of patients with ART-related hepatotoxicity. ULL, upper normal limit; ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitors; ABC, abacavir.

Steatosis and Lipodystrophy in Human Immunodeficiency Virus–Infected Patients

The risk factors of steatosis in non-HIV population such as obesity, diabetes, and alcohol are also valid in HIV patients. Moreover, NRTIs such as d4T, ddC, zidovudine, and ddI may lead to severe microsteatosis along with lactic acidosis in these patients through the impairment of mitochondrial β-oxidation as mentioned previously. In vitro studies have demonstrated that PIs induce inhibition of adipocyte maturation as well as apoptosis and increase lipolysis (146,147,148). PIs have also been associated with hyperlipidemia, IR and consequent hepatic steatosis. IR and the incidence of lipoatrophy are significantly increased in HIV/HCV-coinfected patients compared with those with HIV alone (149). In addition, IR is also significantly associated with steatosis and fibrosis in this group of patients and has an important role in histologic progression of the disease (150). Hepatic steatosis was observed in 40% of HIV/HCV-coinfected patients in a more recent study published by the Johns Hopkins group (151). Although, ART did not seem to have a significant impact on the prevalence of steatosis in their cohort of patients, exposure to d4T was associated with a more than fivefold higher risk of steatosis. These data also suggest that hepatic steatosis is associated with more severe HCV-related liver disease in HIV/HCV-coinfected patients than has been shown previously for HCV-monoinfected patients. PIs and NRTIs acting synergistically are primarily responsible for HIV-related lipodystrophy (HIVLD). Further, several large cohort studies found a

correlation between the duration of therapy with PIs or NRTIs and the development of HIVLD (153,154,155). There is no definite therapy for HIV-related lipodystrophy. In several studies, metformin decreased IR and visceral adipose tissue (156,157). Among the currently available therapies, thiazolidinediones seem to be the best option as they increase insulin sensitivity and subcutaneous adipose tissue while decreasing visceral adipose tissue (158,160). Switching from a PI regimen to another regimen offers very little clinical benefits (160,161,162).

Liver Infections in Human Immunodeficiency Virus–Infected Patients with Severe Immunosuppression

As HIV infection has turned into a well-controlled chronic disease with relatively preserved CD4 counts

after the introduction of ART, the incidence of opportunistic infections has declined substantially. However, severely immunosuppressed HIV patients are susceptible to a variety of opportunistic infections involving the liver. These may be seen in 33% to 78% of autopsy series including patients with AIDS (163,164), and in liver biopsy of almost 90% of HIV seropositive patients with abnormal liver function tests (165). A thorough clinical and diagnostic workup is warranted, which may include liver biopsy with appropriate cultures and staining, provided that less invasive tests are not conclusive.

Viral Infections

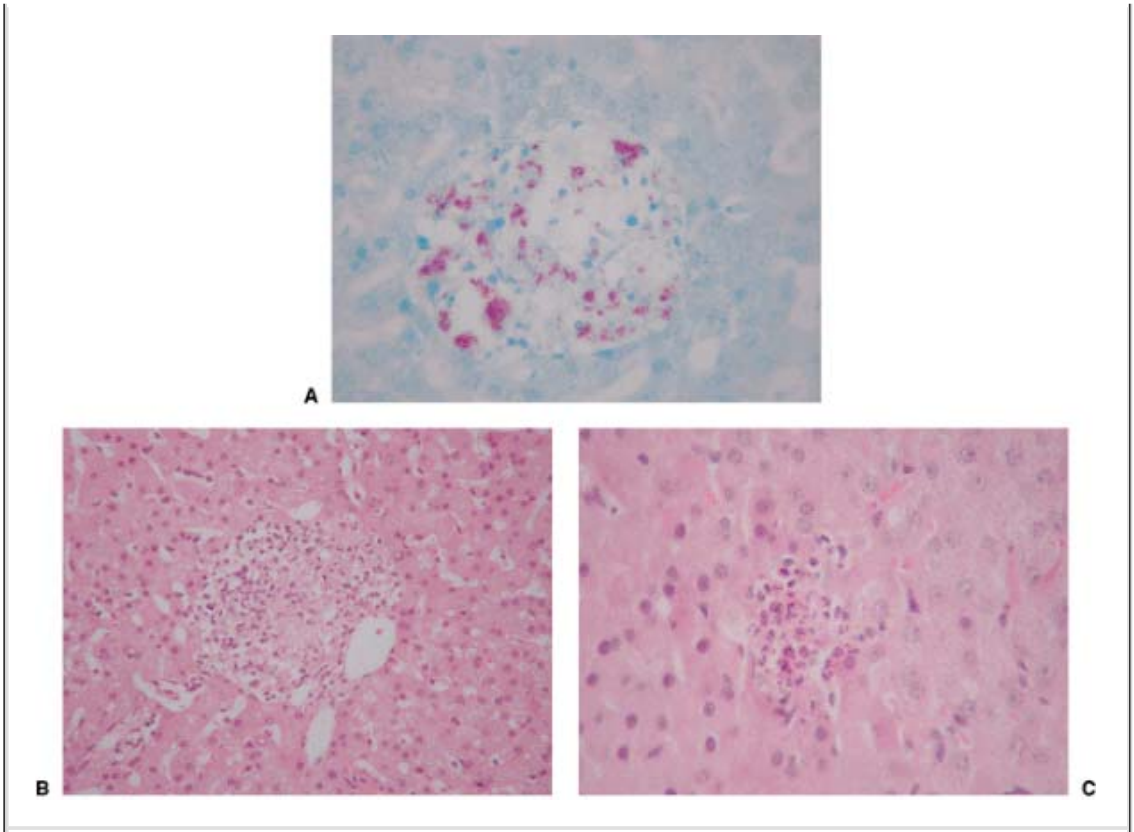
Opportunistic viral infections of the liver are often because of herpes viridae family. Although clinically significant hepatitis secondary to CMV or HSV is rare, CMV is detected frequently in autopsies of severely immunosuppressed patients with CD4 counts less than 100/mm^3 and is often a component of systemic involvement (166). Serum transaminases may be markedly elevated. Liver histology typically shows large intranuclear and cytoplasmic owl's eye inclusions with significant inflammation of portal and periportal regions and sparse necrosis. CMV infects every type of cell within the liver. CMV infection is being detected earlier now owing to pp65 antigen flow cytometry and CMV polymerase chain reaction. In addition, the development of ganciclovir (both intravenous and oral) has reduced the morbidity and mortality of CMV infection (167,168). The major toxicity of ganciclovir and valganciclovir is bone marrow suppression. Resistance can be a problem with ganciclovir that may require switching to foscarnet. The major side effect limiting the use of foscarnet is its nephrotoxicity. Hepatitis secondary to HSV occurs in patients with extensive herpetic ulcers elsewhere. Patients may develop submassive hepatic necrosis with severe transaminitis and liver failure. Pathologically, HSV hepatitis is characterized by multinucleated hepatocytes and Cowdry A intranuclear inclusion bodies that may be differentiated from those of CMV by specific immunohistochemistry. HSV hepatitis may be treated with intravenous acyclovir, ganciclovir or foscarnet. However, hepatitis secondary to either CMV or HSV responds poorly to current treatments. Varicella-zoster virus, EBV, and adenovirus are the other agents responsible for viral hepatitis in patients with HIV.

Mycobacterial Infections

Mycobacterium avium complex (MAC) is the most common opportunistic pathogen

affecting the liver. However, with the introduction of ART the incidence of disseminated MAC infection has declined, and the prognosis has been significantly improved (169,170). Infection with MAC manifests with systemic symptoms and signs, such as fever, abdominal pain, wasting, and biliary obstruction secondary to enlarged lymph nodes at the porta hepatis. It is ordinarily seen in late stage AIDS patients with CD4 count less than 50 cells/mm^3. MAC is detected in 20% to 55% of autopsies and in 10% to 30% of liver biopsies in patients with AIDS (171,172). Severe cholestasis with marked elevations in ALP is typical. However, high transaminases may be the only abnormality. Jaundice is rare. Blood cultures are the most sensitive test for diagnosis of MAC. On the other hand, liver biopsy showing diffuse, poorly formed noncaseating granulomas is necessary for definitive diagnosis of liver involvement. Abundant acid-fast bacilli may be seen on staining (Fig. 51.4), however, liver tissue culture is needed to distinguish between different *Mycobacterium* species. Liver biopsy has been reported to be more sensitive than bone marrow biopsy in diagnosing disseminated mycobacterial infection in AIDS (173). The long-term prognosis is poor with a median survival of only 6 months (174). Combination therapy with a macrolide such as clarithromycin or azithromycin, rifampin, and ethambutol is the most common regimen. Ciprofloxacin or amikacin may be added in more severe cases. Previous data suggests that 12 months of therapy may suffice for patients who are on ART with sustained CD4 counts greater than 100/mm^3 (175). Prophylaxis with azithromycin or clarithromycin is indicated in patients with CD4 counts less than 50/mm^3. Extrapulmonary *Mycobacterium tuberculosis* (MTB) infection involving the liver occurs in 5% to 10% of HIV-related tuberculosis cases and may present with tuberculous liver abscess in severely immunocompromised patients (176). However, ART and rifampicin containing antitubercular therapy have decreased the incidence, recurrence, and mortality rate of tuberculosis (177,178). Tuberculosis is usually because of the reactivation of a latent infection. Liver tuberculosis is associated with fever, abdominal pain, hepatosplenomegaly, and wasting similar to MAC infection. However, as the virulence of MTB is greater than in the other species of *Mycobacterium*, it may infect the patients who have higher CD4 counts, more than 200/mm^3 (179). Cholestasis is seen with significantly elevated ALP and mild increase in bilirubin and transaminases. The specific diagnosis is made by culture and polymerase chain reaction of blood, urine or tissue specimen including liver. Acid-fast bacilli may be seen in the liver histology, which is typically characterized by presence of caseating granulomas. As this infection often occurs in the setting of lesser immunosuppression, granulomas are better formed than those of MAC. The combination of isoniazid, rifampin, ethambutol, and pyrazinamide for 9 to 12 months is the treatment of choice for tuberculosis of the liver. Liver chemistry tests should be closely monitored

during treatment especially in elderly patients. Rarely, liver infections with other mycobacterial species such as *Mycobacterium kansasii*, *Mycobacterium xenopi*, and *Mycobacterium genavense* have also been reported.



▪ **Figure 51.4 A:** An epithelioid granuloma containing *Mycobacterium avium* (Acid-Fast Bacilli stain, ×200). **B:** A microgranuloma in a patient with mycobacterial infection (hematoxylin-eosin stain, ×200). **C:** A microabscess in the liver of a patient with AIDS and CMV infection (hematoxylin-eosin, ×200). AIDS, acquired immunodeficiency syndrome; CMV, cytomegalovirus.

Fungal Infections

Major fungal pathogens infecting the liver are *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Candida albicans*. They are uncommon, and ordinarily seen in the setting of disseminated disease, and in patients with less than 100 CD4 cells/mm^3. They cause cholestasis, and imaging studies demonstrate focal or diffuse lesions in the liver. Liver histology often reveals poorly formed granulomas with minimal inflammatory reaction. Meningitis is the most common manifestation of cryptococcal disease in HIV-infected patients. Liver involvement occurs because of hematogenous spread. The common presenting features are fever and hepatosplenomegaly. Histoplasmosis is particularly common in midwestern United States, Central and South America, and the Caribbean including Puerto Rico. Progressive disseminated histoplasmosis is often the first sign of immunodeficiency in patients with AIDS in endemic areas (180). Constitutional symptoms along with hepatosplenomegaly and lymphadenopathy are the common features. *C. immitis* infection usually occurs in patients infected with HIV who are living in endemic areas such as the Southwestern United States. The disease typically presents with pulmonary involvement; hepatic infection is again secondary to disseminated disease. Systemic infection and liver involvement with candidiasis are quite rare unless the patients are neutropenic. The symptoms are nonspecific, such as nausea,

vomiting, abdominal pain, and hepatomegaly. *C. neoformans* and *H. capsulatum* may be rapidly detected by polysaccharide capsular antigenemia; other causes of fungal hepatitis need special staining and culture of the liver tissue for definitive diagnosis. There are also rare reports of *Aspergillus fumigatus* causing liver abscess and disseminated *Sporothrix schenckii* involving the

liver. Treatment consists mainly of amphotericin B for fungal hepatitis; however, as the relapse rate is high for most of the fungal infections in immunocompromised patients, fluconazole or itraconazole have been suggested as suppression therapy indefinitely in most cases.

Protozoal Infections

Protozoa are rare pathogens causing infections in patients with AIDS. The routine prophylaxis with trimethoprim/sulfamethoxazol (TMP-SMX) has reduced the incidence of *Pneumocystis jirovecii*, *Toxoplasma gondii*, and *Listeria monocytogenes*. In the past *P. jirovecii* (formerly *Pneumocystis carinii*) hepatitis was associated with the prophylactic use of aerosolized pentamidine for pneumocystis pneumonia (181,182). Inhalation failed to provide adequate drug levels to extrapulmonary sites and up to 39% of such patients developed extrapulmonary spread (183). A mixed pattern of elevated liver enzymes is usually seen. Abdominal CT scan may demonstrate diffuse and punctuate calcifications in the liver. Liver biopsy shows foamy nodules that are periportal or diffuse containing numerous Pneumocystis cysts that stain with Gomori's methenamine-silver. Treatment is with intravenous TMP-SMX or pentamidine. *T. gondii* also involves the liver rarely through hematogenous dissemination and may present with granulomatous disease or hepatitis. Diagnosis is made by culture or microscopic examination of Giemsa-stained specimens. TMP-SMX is the drug of choice for toxoplasmosis. Microsporidial infection of the liver is rare and is associated with a rise in bilirubin, mildly raised transaminases, and high ALP levels (184). Light microscopy reveals focal granulomatous and suppurative necrosis, mainly in the portal area, accompanied by characteristic spores. Although there is no established therapy for microsporidial hepatitis, treatment with albendazole may be efficacious for Encephalitozoon intestinalis (185). *Strongyloides stercoralis*, a helminth of the nematode family, may result in a hyperinfection syndrome in the immunocompromised patient. The liver is involved through the hematogenous spread of the larvae from the gastrointestinal tract. *Entamoeba histolytica* may invade the bowel wall and spread to the liver, forming an abscess. Reactivated *Leishmania donovani* is another rare infection of the liver.

Infections of Biliary Tract

The biliary tree including the gallbladder, is a common site of infection in HIV-infected patients with immunosuppression. It may be involved in the form of acalculous cholecystitis or cholangiopathy.

Acquired Immunodeficiency Syndrome Cholangiopathy

AIDS cholangiopathy is a form of secondary sclerosing cholangitis that occurs because of opportunistic infections in advanced stages of HIV infection, particularly in patients with CD4 counts of less than 135/mm^3 (186). Affected patients develop right-upper-quadrant pain, fever, and cholestasis. ALP is often

elevated up to 10 to 20 times above normal. Jaundice is seldom seen as biliary obstruction is usually partial (187,188). There are multiple opportunistic organisms that have been identified in the biliary tree of the patients including CMV, Cryptosporidium, Microsporidia, and less often MAC, *Isospora belli*, and *Enterocytozoon bieneusi*. However, no pathogen is isolated in up to 50% of cases (189). Although liver ultrasonography may show biliary dilatation and magnetic resonance cholangiopancreatography (MRCP) may demonstrate characteristic findings of cholangiopathy, endoscopic retrograde cholangiopancreatography (ERCP) is more sensitive and has an advantage of tissue biopsy and sphincterotomy when necessary. Cello (190) has described four patterns of abnormalities shown by ERCP: (i) stenosis of the papilla of Vater with dilated extrahepatic bile ducts, (ii) sclerosing cholangitis, (iii) combined sclerosing cholangitis and papillary stenosis, and (iv) long extrahepatic strictures with or without sclerosing cholangitis. Duodenal and papillary biopsies and biopsies from inside the biliary ducts may be sent for both culture and pathologic examinations. Endoscopic sphincterotomy may provide benefit for the symptomatic patients with papillary stenosis (188,190). Prognosis depends on the degree of underlying HIV-related immunosuppression rather than the hepatobiliary disease (191). ART has significantly improved the mortality of patients with AIDS cholangiopathy (192).

Acalculous Cholecystitis

Acalculus cholecystitis usually occurs in later stages of HIV infection at CD4 counts of less than 50/mm^3 (188). Concurrent cholangitis is commonly seen (193). Patients usually present with abdominal pain, fever, nausea, and vomiting. Jaundice is rare, but ALP and γ-glutamyl transpeptidase (GGT) are markedly elevated in most cases. In this condition, the gallbladder wall appears thickened and edematous on imaging without any gallstones. Pericholecystic fluid can be seen. Gallbladder histology often reveals an opportunistic organism. The spectrum of responsible pathogens is similar to that identified in AIDS cholangiopathy. Complications of acalculous cholecystitis include perforation and peritonitis. Cholecystectomy can be performed in most patients; in the remaining cases,

CT or ultrasonography-guided percutaneous cholecystostomy may be an option to decompress the gallbladder (194).

Hepatic Mass Lesions in Human Immunodeficiency Virus

The most common hepatic neoplastic mass lesion is Kaposi sarcoma (KS) caused by human herpesvirus 8 (HHV-8). The incidence of KS in HIV-infected patients may be 10% to 20%, more than 100,000 times that in the general population. However, its incidence in AIDS continues to decrease and the lesions may respond to ART only. Among HIV-infected individuals, KS occurs predominantly in homosexual and bisexual men. Despite the presence of hepatic KS in approximately one third of all patients with cutaneous involvement, it is seldom found on antemortem biopsy. Patients may present with abdominal pain and hepatosplenomegaly with an elevated ALP level, but most commonly it is asymptomatic. There is no specific finding on CT scan, however, the lesions are hypo-attenuated and enhance after a bolus of intravenous contrast. The tumor appears to be a vascular proliferation characterized by the presence of spindle cells, vascular channels, and a mixed cellular infiltrate on histology. B-cell lymphoma is the second most common neoplasm in patients with HIV infection

after KS. It is controversial whether the incidence of non-Hodgkin lymphoma has changed since the introduction of ART. Non-Hodgkin lymphomas in patients with AIDS are often B-cell phenotype with high grade and advanced stage, and predominantly extranodal involving most frequently the central nervous system and the gastrointestinal system (195,196). They appear to be a late manifestation of HIV disease with rates rising directly with the duration of infection. Liver is involved in approximately 10% of cases. The CD4 count is usually less than 200/mm^3. Primary hepatic lymphoma usually presents with multiple masses and involvement of other abdominal organs or lymph nodes. Persistent fever, tender hepatomegaly, and mildly abnormal liver chemistry tests combined with an elevated lactate dehydrogenase level may give a clue to the diagnosis. The lesions appear hypodense on both noncontrast and contrast-enhanced CT imaging. Diagnosis can be established by laparoscopic or CT-guided biopsy. However, as bone marrow involvement is frequent, a bone marrow aspirate or biopsy should be considered before a more invasive liver or retroperitoneal lymph node biopsy. AIDS-associated lymphomas have generally an aggressive course and respond poorly to treatment. Bacillary peliosis hepatis is a vascular lesion caused by *Bartonella henselae*, a fastidious gram-negative bacillus. It usually occurs in patients with CD4 counts less than 200/mm^3 (197). Peliosis hepatis may be found incidentally on liver biopsy or occasionally can present with abdominal pain, hepatosplenomegaly, liver failure, and portal hypertension in extensive cases. Systemic symptoms such as fever, anemia, and cutaneous lesions of bacillary angiomatosis may be observed. Laboratory analysis may reveal elevated ALP level and prothrombin time. Abdominal CT scan displays multiple, small, low-attenuated lesions scattered in the liver parenchyma. Biopsy shows cystic blood-filled spaces within the liver that are usually a few millimeters in size. They are associated with fibromyxoid stroma containing clumps of bacilli that can be detected by Warthin-Starry stain. Erythromycin is the drug of first choice for the treatment of peliosis. Other options include clarithromycin, tetracycline or doxycycline. Liver abscess secondary to mycobacterial infections and HCC that may be seen in the setting of HCV or HBV coinfection are other mass lesions observed in HIV-infected patients. The incidence of mycobacterial liver abscess seems to be decreasing because mycobacterial infection is not as common with the introduction of ART and effective antimycobacterial therapy. On the other hand, the incidence of HCC in HIV-infected patients seems to be increasing because of improved survival with ART and the rising incidence of end-stage liver disease because of HCV or HBV coinfection and alcohol abuse (198).

Liver Transplantation in Human Immunodeficiency Virus

The prognosis of infection because of HIV has improved dramatically over the last few years after the initiation of antiretroviral therapy. The outcome of liver disease is more severe in HIV-infected patients and they have a high liver related mortality (86,199,200). Until recently, the presence of AIDS was an absolute contraindication to liver transplantation and asymptomatic HIV infection was a relative contraindication (201). In recent years, liver transplantation is fast becoming the cornerstone in management of end-stage liver disease in patients with HIV infection. In the pre-ART era the outcome of liver transplantation in HIV-infected patients was poor with a high incidence of opportunistic infections resulting in higher mortality (202,203,204). However in recent years, with widespread use of ART, the outcome after liver transplantation has improved significantly. In a multicenter analysis, 24 HIV patients with end-stage liver

disease who underwent liver transplantation were compared with 5,225 HIV-negative patients from the United Network of Organ Sharing (UNOS)

database (205). In HIV-infected patients the etiology of liver disease was chronic hepatitis C in 63%, chronic hepatitis B in 29%, and acute liver failure in 13% of subjects. The cumulative survival among HIV-positive recipients was similar to that among age and race comparable HIV-negative recipients (P = 0.365). At 12, 24, and 36 months after transplant, survival was 87.1%, 72.8%, and 72.8% respectively among HIV-positive patients versus 86.6%, 81.6%, and 77.9% among HIV-negative patients. However, in the HIV/HCV-coinfected group, the survival was worse compared with the HCV-monoinfected group. In HIV-positive patients the 1-, 2-, and 3-year survival after transplant was 80%, 57%, and 57% respectively, compared with 87%, 81%, and 77% respectively, in the HIV-negative group. In this study, worse outcome was associated with posttransplant antiretroviral intolerance, a CD4 count of less than 200 cells/μL, HIV viral load greater than 400 copies/mL, and hepatitis C coinfection. In another series from King's College Hospital, London, 14 HIV-infected liver allograft recipients were evaluated (206). HCV was the etiology of liver disease in seven patients, HBV in four patients, alcohol in two patients, and acute liver failure in 1 patient. In the non-HCV group (n = 7), all patients were still alive with a survival range of 668 to 2,661 days after transplantation, none experienced HBV recurrence, and graft function was normal in all patients. However, five of seven HCV-infected patients died after transplantation at 95 to 784 days (median 161 days); most deaths were because of complications of recurrent HCV and sepsis. This study demonstrated a poor outcome in the HCV and HIV-coinfected patients. Therefore, the limited data so far suggests that the survival in the HIV-infected patients undergoing liver transplantation is comparable to their HIV-negative counterparts. However, the outcome in the HIV/HCV-coinfected patients seems to be worse in compared with the HCV-monoinfected patients.

Therefore, recent findings suggest that survival of HIV-positive liver transplant recipient does not differ from that of HIV-negative recipients and HIV should not be a contraindication to liver transplantation, provided patients meet the currently practiced inclusion and exclusion criteria for listing. In 2003, an NIH sponsored prospective multicenter study was designed to evaluate various issues in liver and kidney transplantation in people with HIV disease. One hundred and twenty-five liver transplant recipients are to be evaluated, and all patients are to be followed for 2 to 5 years. The primary endpoints are patient and graft survival. Secondary endpoints are markers of HIV disease such as opportunistic infections, HIV viral load, and CD4+ cell counts; influence of HCV, HBV, and herpes virus coinfection on graft survival and rejection; and interaction between immunosuppressive drugs and antiretroviral agents. Overall, the study aims to provide patients and clinicians with information regarding the HIV-specific risks of transplantation, to provide clinicians with information necessary to manage immunosuppressive and ART medication together, and to understand underlying basic science mechanisms that explain patient outcomes so that clinical management may be adjusted to maximize these outcomes. All HIV-infected patients with end-stage liver disease should be considered as candidates for liver transplantation provided they do not have advanced HIV disease. Those with severe immunosuppression (<100 CD4 cells/μL) should be treated with ART to control viral replication before they are evaluated for transplantation. Patients with good response to ART, but with prior AIDS-related opportunistic infections and neoplasm have higher risk of relapse while on immunosuppressive medication

after transplantation. There are some important pharmaco-kinetic drug interactions between PIs and NNRTIs and immunosuppressive agents like tacrolimus and cyclosporin. PIs can increase the levels of tacrolimus and cyclosporin, whereas NNRTIs can reduce their levels. Therefore, optimizing the dose of immunosuppressive medication to prevent toxicity secondary to drug interactions with ARV agents, use of ART to suppress HIV RNA, and maintaining CD4 counts greater than 200 to decrease risk of opportunistic infections are some ways to improve survival after liver transplantation in the HIV-infected patients. Liver transplantation in HIV-positive patients is promising, and further prospective long-term studies such as the NIH trial are needed to firmly establish its role in management of these patients.

Annotated References

Sherman KE, Rouster SD, Chung RT, et al. Hepatitis C virus prevalence among patients infected with Human Immunodeficiency Virus: a cross sectional analysis of the US adult AIDS Clinical Trials Group. *Clin Infect Dis* 2002;34:831–837.

In this analysis, a large sample of 1687 HIV-infected patients was studied, They demonstrated that Hepatitis C virus has emerged as an important etiologic agent of liver injury and failure in patients infected with human immunodeficiency virus (HIV), and the prevalence is related to the risk behavior. They also showed that high virus loads and genotype 1 prevalence may be important to interferon-based antiviral response rates among coinfected patients.

Sulkowski MS, Thomas DL, Chaisson RE, Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis B or C virus infection. *JAMA* 2000;283:74–80.

It is well known that antiretroviral medication causes hepatotoxicity. When severe, this often results in discontinuation of treatment, putting the patient at risk for advancement HIV disease. The authors have shown that severe hepatotoxicity occurs in only 10% of patients, but the risk is increased in patients receiving ritonavir and in patients who have coinfection with hepatitis B or hepatitis C.

Torriani FJ, Rodriguez-Torres M, Rockstroh JK, et al. Peginterferon alfa 2a plus ribavirin for chronic hepatitis C virus infection in HIV infected patients. *N Engl J Med* 2004;351:438–450.

In this study a total of 868 persons who were infected with both HIV and HCV and who had not previously been treated with interferon or

ribavirin were randomly assigned to receive one of three regimens: peginterferon alfa-2a plus ribavirin, peginterferon alfa-2a plus placebo, or interferon alfa-2a plus ribavirin. The authors demonstrated that among patients infected with both HIV and HCV, the combination of peginterferon alfa-2a plus ribavirin was significantly more effective than either interferon alfa-2a plus ribavirin or peginterferon alfa-2a monotherapy.

Chung RT, Anderson J, Volberding P, et al. Peginterferon alfa -2a plus ribavirin versus interferon alfa 2a plus ribavirin for chronic hepatitis C in HIV

co-infected persons. *N Engl J Med* 2004;351:451–499.

In the AIDS Clinical Trials Group (ACTG) study viral response was better in the peginterferon and ribavirin group rather than the IFN group. Treatment with peginterferon and ribavirin was associated with a significantly higher rate of SVR than treatment with standard IFN and ribavirin. The study showed that independent factors associated with higher SVR were genotype non-1 infection, no prior intravenous drug use, and an undetectable HIV RNA at baseline.

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Chapter 52
Granulomas of the Liver

James H. Lewis

Key Concepts

- Hepatic granulomas are currently found in 5% to 10% of patients in liver biopsy series, and represent a localized inflammatory response to a variety of infectious, immunologic, drug-induced, neoplastic, and nonmicrobial chemical and foreign body irritants. Both genders and all ages can be affected.
- While dozens of causes have been reported worldwide, the most common are sarcoidosis and tuberculosis (TB). More than 60 drugs have been associated with a granulomatous reaction, most often causing a hypersensitivity reaction (e.g., allopurinol, sulfonamides, phenylbutazone, phenothiazines, carbamazepine).
- Two main types of granulomas are found—lipogranulomas that form around fat droplets associated with ingestion of mineral oil, waxes, and other lipid materials, and epithelioid granulomas that are usually formed in response to a hypersensitivity reaction. A special form of epithelioid granuloma is the fibrin-ring granuloma seen with Q fever, allopurinol, and a few other causes.
- Granulomas may be found as a coincidental lesion or may serve to confirm a variety of infectious or other disorders associated with their development. They may be the only clue to the presence of certain infectious or neoplastic diseases, and occasionally are found as part of a fever of unknown origin (FUO). Approximately 10% are considered "idiopathic" with no specific etiology identified, even by utilizing modern serologic and immunohistochemical methods.
- Although biochemical tests are generally nonspecific, jaundice is unusual with most causes of granulomas, and should prompt a search for other etiologies. Certain histopathologic clues to the etiology of granulomas continue to be emphasized, such as sarcoidosis having multiple noncaseating granulomas of different ages; TB having caseating granulomas; many drugs and parasites being associated with surrounding eosinophils; and fibrin-ring granulomas suggesting Q fever among a few other causes.
- Treatment of hepatic granulomas depends on the etiology and the degree of symptomatology. Chronic cholestatic liver disease leading to biliary cirrhosis may be seen in a small percentage of patients with sarcoidosis. In general, however, granulomatous hepatic involvement by most causes is nonprogressive. Drug-induced causes often resolve after the offending agent is withdrawn.

Granulomas are focal collections of epithelioid cells, including macrophages, mononuclear and other inflammatory cells that may fuse together to form multinucleated giant cells, usually in response to a variety of infections, immunologic and neoplastic disorders, drugs, and nonmicrobial chemical irritants (1,2). They are the end result of a complex interplay of inflammatory and immunologic factors (3), depending on the nature of the infectious or antigenic stimulus. The liver is a common location for granulomas due to its prominent blood supply that exposes most drugs, infectious organisms, and other materials to its large numbers of reticuloendothelial (Kupffer) cells. When found, granulomas usually prompt a search for a specific etiology from a list that has grown to include dozens of causes, as will be discussed. Hepatic granulomas are present in approximately 5% of large modern-day liver biopsy series, with a range of 1% to 15%, varying by geographic location and disease prevalence (4,5,6,7,8,9,10,11,12,13,14,15,16,17,18) (Table 52.1). This also appears to apply to pediatric populations from around the globe (19,20,21,22,23). Granulomas may be an anticipated or incidental finding, reflecting either a primary hepatic disorder (approximately 5%), or seen as part of a systemic disease process with hepatic involvement(70%–75%), including fever of undetermined etiology (4,24,25,26,27).

Both foreign body type granulomas (nonimmunologic) and hypersensitivity-mediated granulomas can be seen in the liver (28). Granulomas are most likely to be seen when macrophages drawn to the site of acute inflammation are unable to clear antigens and inflammatory byproducts. As a result, monocytes are attracted from the blood and bone marrow and form tissue epithelioid histiocytes that fuse together to become multinucleated (Langhans-type) giant cells (29,30,31,32). Such cells lose their phagocytic function and develop secretory properties (33), such as sarcoid granulomas producing angiotensin-converting enzyme (34). The cytokine network and immunology associated with granuloma formation has been reviewed in detail by James (3).

A variety of disorders are associated with a granulomatous histological response that can involve one or more organ systems (3). Infections are the most common cause of disseminated granulomas, and previously unrecognized etiologic agents have been identified by improved diagnostic techniques (35). Nevertheless, granulomas often still present a diagnostic challenge to the clinician.

Men and women have been nearly equally represented in several large series of granulomas, although women, not unexpectedly, predominate when primary biliary cirrhosis (PBC) accounts for a significant percentage of cases (16). The ages of adult patients with hepatic granulomas generally have been in the range of forties to fifties, but

children are affected as well (19,20,21,22,23).

Pathophysiology of Granulomas

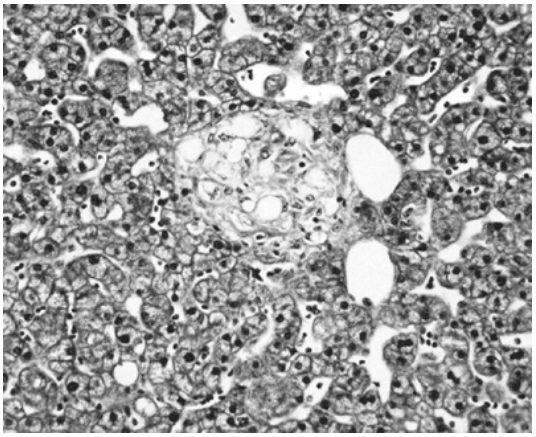
Histologically, two main types of granulomas are described—lipogranulomas and epithelioid granulomas.

Lipogranulomas are composed of loose aggregates of lymphocytes and macrophages surrounding lipid droplets, and are secondary to ingestion of mineral oil or other lipid material (36,37) (Fig. 52.1). They may also be seen in fatty livers, possibly originating from parenchymal fat being transported to portal tracts (38). In a series of 44 nonfatty liver cases reported by Dinscoy et al. (39), lipogranulomas were attached to or were in close association with the walls of hepatic venules. The lipogranulomas in their series were most likely a reaction to absorbed mineral oil or other saturated hydrocarbon foodstuffs. Lipogranulomas comprise a relatively smaller percentage of all granulomas, ranging up to 26% (16), but were described as having nearly tripled in incidence between the early 1950s and the late 1970s in one series (39). Among autopsy specimens, lipogranulomas have been found in 48% often in association with lipogranulomas in the spleen (36). They may be discovered incidentally and are generally of little, if any, clinical significance (37,40). However, reports of venous outflow obstruction (41) and a case of prolonged fever associated with lipogranulomas caused by paraffin oil have been published (42).

Epithelioid granulomas are the predominant histologic form in most series. The name is derived from

P.1427
P.1428
P.1429

their polygonal shape and abundant cytoplasm (18) (Fig. 52.2). They generally develop in response to delayed hypersensitivity reactions (3). Cytokines mediating the Th1 immune response attract $CD4^+$ and $CD8^+$ T lymphocytes and fibrocytes that form the periphery of many granulomas. Activation of the Th2 immune response occurs as a result of antigenic sensitization, leading to necrosis or caseation, depending on the nature of the antigenic stimulus (43,44,45).



▪ **Figure 52.1** Lipogranuloma secondary to mineral oil (hematoxylin-eosin, ×250). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

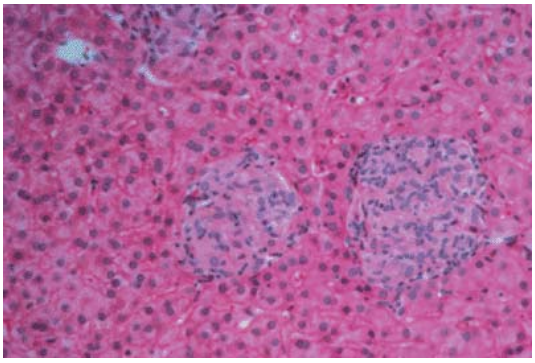
Table 52.1. Causes of Hepatic Granulomas by Geographic Location

Author (Ref) location	Years covered	No. of cases (% of liver bx)	Etiology(%)					
			TB	Sarcoid	DILD	Misc.	PBC	Idiopathic
Mir-Madjlessi (5) Cleveland	1966–1971	*n* = 50 (2.4% of 2,086)	10	22	Up to 18	14—32 Histoplasmosis 12 Cirrhosis 8 Lymphoma 6	–	36
Neville (6) London	1965–1975	*n* = 138	2.5	54	—	17 CLD 9	19	10
Vilaseca (7)	1971–	*n* =	28	18	—	47	—	7.4

Spain	1977	107				Mediterrean fever 12 Infections 15		
McMaster (8) S. Carolina	1969–1978	*n* = 95 (6% of 1,500)	8.5	33	29	12 Visceral larva migrans 2 Fungal 1 Neoplasm 3	—	12
Cunningham (9) Glascow	1970–1979	*n* = 77	10	10	—	48 CLD 15 Bile duct obstruction 9 Neoplasm 8	Excluded	11–31
Anderson (10) Australia	1968–1984	*n* = 59	7	12	7	45 Q fever 5 CLD 20 Neoplasm 8 Biliary tract disease 5	Excluded	29
Sartin (11) Mayo Clinic	1976–1985	*n* = 88	3	22	6	25 Histoplasmosis 4	6	50
McCluggage (12) N. Ireland	1980–1992	*n* = 163 (4%)	2	18	1.5	14 Psoriasis 4 Crohn disease 1.5 CLD 1.5	55	11
Sabharwal (13) India	1985–1995	*n* = 51 (4% of 1,234)	55	—	—	33	—	12
Guglielmi (14) Italy	1989–1994	*n* = 15 (1%)	7	15	20	58 HBV or HCV 20	—	—
Voigt (15) France	1984	*n* = 73	—	33	20	47 Q fever 20 Hodgkin's disease	—	—
Gaya (16) Scotland	1991–2001	*n* = 63 (4% of 1,662)	5	11	9.5	39 HCV 9.5 AIH 6.3 Hodgkin's disease 6.3	24	11
						Resolving obstruction 3		—
Satti (17) Saudi Arabia	1990	*n* = 59	32	—	3.5	65 Schistosomiasis 54 Brucellosis 6	—	—

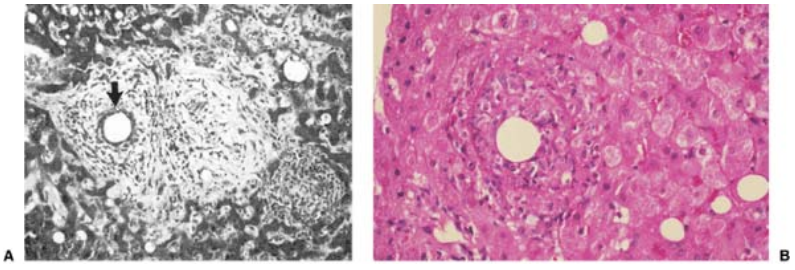
Reynolds (18) University of Southern California	1990	*n* = 169	27	29	1.2	28 leprosy 5.3 Brucellosis 5.3 Hodgkin's disease 4 Q fever 3,5	—	15

% of liver biopsies, the frequency of finding granulomas in the series of liver biopsies being reported; TB, tuberculosis; sarcoid, sarcoidosis; DILD, drug-induced liver disease; Misc., miscellaneous causes (*%of total number of granulomas in the series listed under miscellaneous); PBC, primary biliary cirrhosis; idiopathic, cause not established; —, not listed; CLD, chronic liver disease (not specified); AIH, autoimmune hepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus.



▪ **Figure 52.2** Noncaseating epithelioid granuloma characterized by giant cell transformation (hematoxylin-eosin, ×300). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

A distinctive type of epithelioid granuloma is characterized by a *fibrin ring* enclosing a vacuolar clear space of necrosis (46). These "doughnut"-shaped granulomas have traditionally been associated with Q fever (47) (Fig. 52.3A) and allopurinol toxicity (Fig. 52.3B) (48). However, this pathologic appearance is not limited to these causes, with several other infections and etiologies having been described (49,50,51,52,53) (Table 52.2).



▪ **Figure 52.3** Fibrin-ring granulomas. **A:** Secondary to Q fever. A fat globule is surrounded by a ring of fibrin (*arrow*) (hematoxylin-eosin, ×160). **B:** "Doughnut" type of fibrin-ring granuloma associated with allopurinol toxicity (hematoxylin-eosin, ×150). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Table 52.2. Causes of Fibrin-Ring Granulomas

Hodgkin's disease
Giant cell arteritis
Hepatitis A
Visceral leishmaniasis

Epstein-Barr viral infection
Toxoplasmosis
Boutonneuse fever (*Rickettsia conorii*)
Systemic lupus erythematosus
Staphylococcal infection
Q fever

Etiology of Granulomas

Depending on the geographic locale, the causes vary according to the diseases encountered. For example, in India, most granulomas are due to tuberculosis (TB) (13); in northern Europe, PBC tends to predominate (16); in the midwest of the United States, histoplasmosis is seen more often (5,19); schistosomiasis in the Middle East and the Tropics (17); mediterranean fever in Spain (7); brucellosis and Q fever in sheep and cattle raising regions and in slaughterhouse workers (54,55). In most series, infectious disorders are the leading causes of hepatic granulomas (56), consistent with the notion that granulomas form in response to intracellular pathogens that trigger active cell-mediated immunity, or to the persistence of nondegradable (foreign body) products (3). Table 52.1 lists several representative series of hepatic granulomas from diverse geographic areas, which attest to their spectrum of causes. The etiopathogenesis of hepatic granulomas often depends on the extent to which a specific diagnosis is sought, although no cause was identified in up to half of cases in earlier series. However, with the advanced diagnostic testing currently at

our disposal, including serologic tests and the use of polymerase chain reaction (PCR) analyses for a variety of infectious agents, the percentage of truly "idiopathic" (unexplained) granulomas has been reduced; in more recent series to about 10% (9,19). A complete listing of the causes of hepatic granulomas is given in Table 52.3.

Table 52.3. Causes of Hepatic Granulomas[a]

Infections
- Bacteria
  - Brucellosis
  - Catscratch disease (*Bartonella henselae*)
  - *Yersinia enterocolitica*
  - Melioidosis
  - Nocardiosis
  - Tularemia (*Pasteurella tularensis*)
  - Salmonellosis
  - *Staphylococcus*
- Mycobacteria
  - *Mycobacterium tuberculosis*
  - *Mycobacterium avium* complex
  - *Mycobacterium leprae* (Léprosy, Hansen's disease)
  - Atypical mycobacteria
  - Bacillus Calmette-Guérin vaccination
- Fungi
  - Histoplasmosis (*Histoplasma capsulatum*)
  - Coccidioidomycosis (*Coccidioides immitus*)
  - Candidiasis
  - Blastomycosis
  - Aspergillosis
  - Mucormycosis
- Parasites
  - Schistosomiasis
  - Toxocariasis, visceral larva migrans (*Toxocara canis*)
  - Visceral leishmaniasis (*Leishmania donovani*; kala azar)
  - Strongyloidosis
  - Fascioliasis
  - Giardiasis
- Chlamydia

Psittacosis (*Chlamydia psittaci*)
- Rickettsiae
  - Q fever (*Coxiella burnetti*)

Boutonneuse fever (*Rickettsia conorii*)
Scrub typhus (*Rickettsia tsutsugamushi*)
Spirochetes
Syphilis (*Treponema pallidum*)
Viruses
Hepatitis A
Hepatitis B
Hepatitis C
Cytomegalovirus
Mononucleosis (Epstein-Barr virus)
Varicella
Neoplasms
Hodgkin's disease
Lymphoma
Hairy cell leukemia
Renal cell carcinoma
Drugs (see Table 52.9)
Metals
Beryllium
Copper sulfate
Gold
Aluminum
Thorotrast (thorium dioxide)
Foreign materials
Silica
Talc (IV drug use, glove powder)
Silicone (spallation of dialysis tubing, ball-valve prostheses)
Mineral oil
Barium sulfate
Suture material
Cement, mica dust
Polyvinyl pyrrolidone
Miscellaneous causes
Sarcoidosis
Jejuno-ileal bypass
Primary biliary cirrhosis
Primary sclerosing cholangitis
Chronic biliary obstruction
Hypogammaglobulinemia
Wegener granulomatosis
Chronic granulomatous disease
Giant cell arteritis
Rheumatoid arthritis
Crohn's disease
Post–liver transplant rejection, recurrent disease
Idiopathic (no cause established)
Factitious granulomas (quinine)[b]

[a]These are based on references 18,22,29,57–59.
[b]From (60) Schlegel A. Factitious granulomatous hepatitis? *Am J Med* 2004;116:500–501.

Clinical Consequences of Hepatic Granulomas

Granulomas draw attention on the basis of the clinical setting in which they arise; either as part of an acute generalized hypersensitivity reaction or infection, during the evaluation of fever of unknown origin (FUO), or as part of the workup for abnormal liver-associated enzymes (usually alkaline phosphatase, γ-glutamyltransferase, or hyperglobulinemia). In a Mayo Clinic series (10), 74% of patients had unexplained symptoms (including fever) present for a mean of 19 months, with the remainder of granulomas being discovered on liver biopsy for abnormal results on liver function tests. While granulomas are often associated with a classic inflammatory response, significant hepatocellular dysfunction is unusual; hence the term "granulomatous hepatitis" (5,24) is less commonly applied. The term favored by most authorities is "hepatic granulomas" as used by my predecessor of this

chapter (57,58) and others (59,61). Despite the relative absence of overt hepatic dysfunction with most granulomas, several pathologically significant lesions are described in association with granulomatous involvement (Table 52.4).

Table 52.4. Clinicopathologically Significant Hepatic Lesions Seen with Granulomas

Pathologic lesion	Causative granulomas (Ref)
Veno-occlusive disease	Lipogranulomas (41)
Chronic cholestasis	Sarcoidosis (62,63),
Vanishing bile duct syndrome	Sarcoidosis, PBC (64)
Portal hypertension	Sarcoidosis, PBC, schistosomiasis (65,66)
Granulomatous hepatitis[a]	Drugs, neoplastic/immunologic disorders, infections (5,24,25,26,27)

[a]Defined as fever, weight loss, abdominal pain, myalgias, arthralgias.
PBC, primary biliary cirrhosis.

Clinical Evaluation of Hepatic Granulomas

The extent to which a specific cause of granulomas in the liver is sought often corresponds to the frequency with which certain diagnoses are found in various geographic locales. A patient's travel history, drug history, occupation, the presence of household pets, and proximity or exposure to domesticated farm or feral animals may provide useful clues as to the diagnosis (Table 52.5).

Table 52.5. Clinical and Historical Clues to the Cause of Hepatic Granulomas

Historical due	Possible cause
Exposure to sheep, cattle, etc.	Q fever, brucellosis
Cat bites or scratches	*Bartonella henselae*
Exposure to puppies	Visceral larva migrans
Slaughterhouse workers, veterinarians	Q fever, brucellosis
Miners, atomic energy plant or ceramics workers	Beryllium
Swimming, wading in streams	Schistosomiasis
Exposure to wild rodents, dogs	Rickettsioses
HIV/acquired immunodeficiency syndrome; sexually transmitted diseases,	*Mycobacterium avium*, *Mycobacterium tuberculosis*, toxoplasmosis, syphilis
Exposure to parrots and other birds	Psittacosis
Exposure to dog ticks	Boutonneuse fever (*Rickettsia conorii*)
IV drug use	Talc
BCG inoculation	BCG

Hemodialysis with silicon tubing or ball-valve prosthesis	Silicone spallation
Jejunoileal bypass for obesity	Lipogranulomas
Chrysotherapy for rheumatoid arthritis	Gold
Mineral oil ingestion	Lipogranulomas
Vineyard workers	Copper sulfate toxicity
Skin lesions	Leprosy, sarcoidosis, syphilis
Fever	TB, sarcoid, most infections, drugs causing hypersensitivity[a]
Living in disease-endemic areas	Leprosy (Mexico, China, South America); TB (worldwide, HIV-positive); histoplasmosis (eastern, midwest United States); coccidioidomycosis (southwest United States); melioidosis (South East Asia); schistosomiasis (Tropics)

[a]Excludes primary biliary cirrhosis parasites
sHIV, human immunideficiency virus; BCG, bacillus Calmette-Guérin; TB, tuberculosis.

Laboratory studies–biochemical markers associated with hepatic granulomas are nonspecific (58). Elevated levels of alkaline phosphatase or γ-glutamyltransferase may give a clue as to the presence of granulomatous disease, but by themselves are nondiagnostic, and may also signify cholestatic disorders. Clinical jaundice is unusual in TB and other infections, but may be seen in other causes, including the uncommon chronic cholestatic lesion of sarcoidosis. (62). Hyperglobulinemia may be present in TB and sarcoidosis. Other commonly employed laboratory tests that can be performed include angiotensin-converting enzyme levels for suspected sarcoidosis, antimitochondrial antibody for suspected PBC, and rapid plasmin reagin (RPR) for syphilis.

Serologic tests are available for a wide variety of infections including Q fever, mononucleosis, cytomegalovirus, Brucella, syphilis, hepatitis A, B, and C, psittacosis, among others. The reader is referred to other sources describing the most accurate diagnostic modalities for infectious causes of granulomas (35) (Table 52.6).

Additional diagnostic studies include *ova and parasite* examination of the stool to uncover schistosomiasis (the organisms), *Toxocara*, or other parasites. Uveitis

revealed on *slit lamp examination* of the eye may be a clue to the presence of sarcoidosis. A *chest x-ray* may reveal changes suggesting TB, sarcoid, Hodgkin's disease (HD), and so on.

Table 52.6. Specific Diagnostic Tests and Treatment of the Most Commonly Encountered Infectious Causes of Hepatic Granulomas

Cause	Diagnostic tests	Treatment[a]
Mycobacterium tuberculosis	Positive AFB smear, culture; PCR	Isoniazid, pyrazinamide, rifampin
Mycobacterium avium	Tissue culture, positive AFB smear	Macrolides, azalides, rifabutin
Mycobacterium leprae	Skin biopsy, positive Fite stain	Dapsone +/- clofazimine, rifampin
Q fever	Serology, PCR, culture	Doxycycline, vaccine for prevention
Brucellosis	Serology; PCR; positive blood, tissue culture	Doxycycline + rifampin or streptomycin

Syphilis	Treponemal serology, PCR	Benzathine or procaine penicillin
Schistosomiasis	Positive serology	Praziquantel
Catscratch fever	Silver stain, tissue culture, PCR	Ciprofloxacin, doxycycline, erythromycin or TMP-SMX
Histoplasmosis	Skin testing, silver stain serology	Azoles (e.g., fluconazole)
Coccidioidomycosis	Precipitin or other antibody tests, skin test, tissue culture	Azoles, amphotericin
Yersinia enterocolitica	Serology, culture	Aminoglycosides, tetracyclines, TMP-SMX or ciprofloxacin
Visceral larva migrans	Larvae in tissue, ELISA	Albendazole
Visceral leishmaniasis	Amastigotes in aspirates, promastigotes in culture, serology	Liposomal amphotericin B
Toxoplasmosis	PCR, serology, tissue isolates	Pyrimethamine + sulfadiazine or clindamycin + leukovorin
Psittacosis	Serology	Tetracycline or doxycycline

[a] Consult Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 6th ed. Philadelphia: Elsevier Churchill Livingstone, 2005; for specific dosages and alternates regimens used for hepatic involvement.
AFB, acid-fast bacillus; PCR, polymerase chain reaction; TMP-SMX, trimethopime-sulfamethoxazole; ELISA, enzyme-linked immunosorbent assay.

Diagnostic *bone marrow* or *lymph node biopsies* are available to help identify granulomas associated with TB, lymphoma, HD, and so on.

Radiologic evaluation may yield clues as to the cause of granulomas, although most granulomas are often too small to be seen on standard imaging. However, the *magnetic resonance imaging* appearance of granulomatous hepatitis includes nodules 0.5 to 4.5 cm in diameter; with caseating granulomas having intermediate to high signal on T2-weighted images and low signal on T1. Noncaseating granulomas were found to have an increased enhancement on arterial phase images that persisted into the late phases. (64) On *ultrasonography*, multiple echogenic lesions 3 to 5 mm in size surrounded by a hypoechoic halo have been described (65). *Diagnostic laparoscopy* is described as revealing a range of liver capsule findings, including exudative, pinpoint, granular, macular, and cord-like features. Findings of TB was most often granular while brucellosis was exudative. (66).

Histopathologic Appearance

Granulomatous reactions may take one of several forms as described by Goodman (67): simple (bland) granulomas without other associated injury; granulomatous hepatitis with hepatocellular injury, including apoptosis and parenchymal inflammation; and granulomatous cholangitis or vasculitis. The pathologic appearance and location of the granulomas may offer important clues to their cause as listed in Table 52.7. Special stains for acid-fast bacillus (AFB) and fungi, the use of polarizing microscopy looking for talc, and immunohistochemical stains for hematogenous malignancies, and so on, may be helpful. Culture of liver biopsy material has a relatively low yield in terms of *Mycobacterium tuberculosis* and other infections, but is often performed, especially in patients with FUO.

Specific Causes of Hepatic Granulomas

The most common causes of hepatic granulomas listed in Table 52.3 are described in the subsequent section.

Sarcoidosis

Sarcoidosis is a chronic multisystem granulomatous disease that is seen in all races and ages, and is the leading cause of hepatic granulomas in the United States. It preferentially affects young African Americans, who have a 10-fold higher prevalence compared to whites, and they tend to have a more severe clinical course (68). The liver is the third most common site of involvement after the lungs and lymphatics. The diagnosis requires the presence of noncaseating granulomas in at least two organs (69). The presence of

systemic symptoms (fever, weight loss, malaise) may occur without pulmonary disease. Hepatic involvement generally does not cause significant morbidity, but a small subset of patients develop progressive cholestatic disease that can lead to cirrhosis and portal hypertension (62,63). Serum alkaline phosphatase levels can become very high (>1000 IU/L), but aminotransferases and bilirubin levels are generally normal.

Table 52.7. Histopathological Clues to the Etiology of Hepatic Granulomas

Large number of granulomas: Sarcoidosis, miliary TB
Lipogranulomas: Fatty liver, mineral oil
Associated bile duct injury: PBC, sarcoidosis
Well-formed granulomas: Sarcoidosis, TB, schistosomiasis, histoplasmosis, coccidioidomycosis, chronic brucellosis, phenylbutazone
Poorly formed granulomas (granulomatous inflammation/necrosis): *Mycobacterium avium*, acute brucellosis, Q fever, many drugs
Caseation (central necrosis): TB
Uniform age of granulomas: Drug injury
Noncaseating granulomas of different ages: Sarcoidosis
Multinucleated giant cells: Sarcoidosis, TB
Inclusions:
Stellate-shaped (asteroid bodies)—sarcoidosis
Lamellar (Schaumann bodies)—sarcoidosis
Fibrin-ring granulomas (central vacuole surrounded by a ring of fibrin) often staining positive for phosphotungistic acid hematoxylin: Q fever, allopurinol, Hodgkin's disease, and other causes listed in Table 52.2
Eosinophils: Drug injury, schistosomiasis, toxocariasis, visceral larva migrans, catscratch disease, Hodgkin's disease, non-Hodgkin's lymphoma[a]
Positive AFB stain: TB, *Mycobacterium avium*
Positive silver stain: Fungi
Foamy macrophages/histiocytes: *Mycobacterium avium*
Foreign body reaction: Talc, silica, schistosome eggs, etc.
Pigment /crystals:
– Black granules—gold, titanium
– Gray-tan birefringent material in reticuloendothelial system—barium
– Coarse, pink-brown granules—thorium dioxide
– Colorless amorphous birefringent crystals—silicone
Location/distribution of granulomas:
– Lobular—drug, sarcoid, brucella
– Portal/periportal—sarcoid, TB, Q fever, drugs
– Periductal—PBC
– Perivenous—mineral oil
– Periarterial—phenytoin

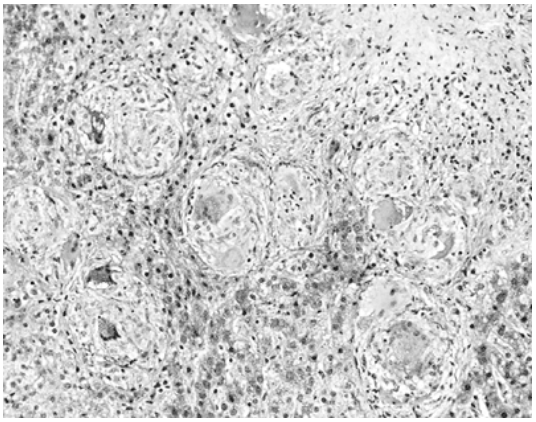
[a]Excludes TB, sarcoidosis.
TB, tuberculosis; PBC, primary biliary cirrhosis; AFB, acid-fast bacillus.

Sarcoid granulomas are compact aggregates of large epithelial cells, often with multinucleated giant cells and a rim of lymphocytes and macrophages, and others with a giant cell, surrounded by lymphocytes (Fig. 52.4). They occur diffusely throughout the liver, but are most prominent in the portal and periportal zones. Klatskin (4) estimated that there were as many as 75 million granulomas in the liver at various stages of maturation. Inclusions in giant cells (Schaumann and asteroid bodies) are characteristic, but not pathognomonic (70,71). The pathogenesis of sarcoid granulomas involves their transformation from tissue macrophages that are derived from circulating blood monocytes. According to Okabe (72), they are under the control of colony-stimulating factors and possibly vitamin D_3, which promote their proliferation. Their transformation to secretory cells, producing angiotensin-converting enzyme, was described by Gronhagen-Riska et al. (34).

Three stages of development were described by Klatskin (4); early on the granulomas are small, later on they become well-defined, ovoid in shape with associated Kupffer cell hyperplasia, and finally form fibrinoid nodules. Sarcoid granulomas tend to segment as they enlarge, forming multilobulated granulomas (18) that may persist for long periods of time. Confluent granulomas may result in extensive, irregular scarring (73). They are never caseating (in contrast to TB), not to be confused with fibrinoid necrosis that can occasionally be seen (71,73). Tissue eosinophilia is also absent (in contrast to drug-induced granulomas). Bile duct damage may be seen (although less commonly and with less severity than in PBC). Large sarcoid nodules situated in the hilum may produce biliary obstruction. Chronic cholestasis resembling PBC is a well-described but less common clinical outcome (Fig. 52.5) (62,74,75). Progression to frank biliary cirrhosis with portal hypertension (from occlusion of intrahepatic portal vein branches) requiring transplantation is relatively uncommon but the disease may recur in

allografts (76). Moreno-Merlo et al. (77) suggested that cirrhosis and focal fibrosis may be caused by ischemia secondary to primary granulomatous phlebitis of portal and hepatic veins, with portal hypertension occurring

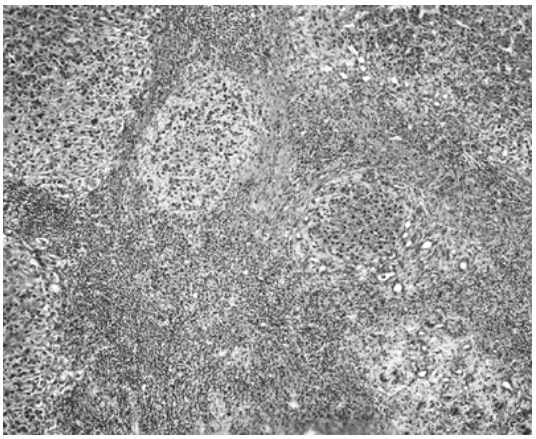
secondary to portal vein thrombosis, as cirrhosis was absent at the onset of variceal bleeding in the two patients they described.



▪ **Figure 52.4** Sarcoidosis with multiple noncaseating epithelioid granulomas having multinucleated giant cells (hematoxylin-eosin, ×150). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Sarcoidosis may be present in association with untreated hepatitis C (78) and can reactivate during interferon therapy (79,80); possibly through the stimulation of the Th1 immune response (81).

In a series of 100 patients with hepatic sarcoid studied by the Armed Forces Institute of Pathology (AFIP) (71), the volume of granulomas was estimated from <1% to 90%; 99% of which were noncaseating. Three biochemical patterns were found; cholestatic in 58% (nearly half of whom had bile duct lesions similar to PBC or primary sclerosing cholangitis [PSC]); necroinflammatory in 41% (with spotty necrosis and/or chronic portal inflammation), and vascular in 20% (with sinusoidal dilatation and nodular regenerative hyperplasia). Ductopenia was found in 37 of 58 individuals with chronic cholestasis. Another 12 had acute cholangitis changes without clinical evidence of ductal obstruction. Fibrosis was seen in 21 patients (periportal in 13, bridging in 2, and cirrhosis in 6).



▪ **Figure 52.5** Chronic cholestasis of sarcoidosis with micronodular biliary cirrhosis (hematoxylin-eosin, ×60). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

The therapeutic options for sarcoidosis have been reviewed by others (68). Treatment of hepatic granulomas is usually administered only when the patient has systemic symptoms from granulomatous hepatitis or cholestatic liver disease, or other symptomatic organ involvement (69). There is no evidence that corticosteroids prevent progression of hepatic disease in asymptomatic individuals (82), although alkaline phosphatase levels may decline. Granulomas may heal without a trace (71). In a series by Gottlieb et al. (83), patients with hepatic involvement had a three-fold higher risk of relapse after a course of steroids compared to those without liver disease. Ursodiol may be beneficial in patients with the cholestatic form of the disease (84,85).

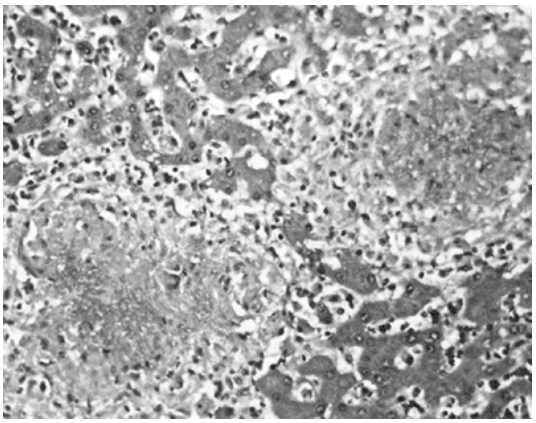
Mycobacterium Tuberculosis

Granulomas are the most common histologic feature of TB involving the liver, but a wide spectrum of nonspecific histopathologic lesions may be present (86). Granulomas are seen in approximately 20% of patients with

pulmonary TB (depending on how many sections are examined) (87) and are seen nearly universally in those with miliary TB (86). Isolated hepatic involvement is also well described (88). As many as two thirds of patients with acquired immunodeficiency syndrome (AIDS) have extrapulmonary involvement that frequently involves the liver (89) (as described in the subsequent text).

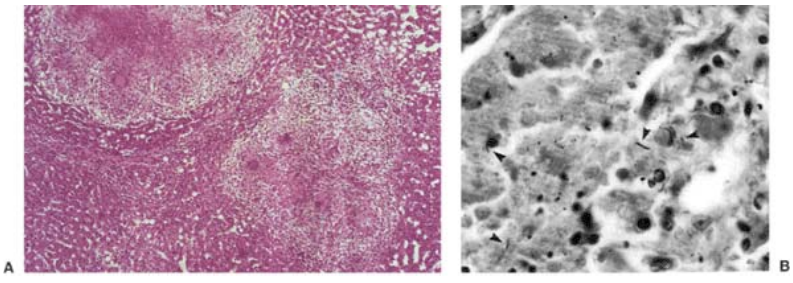
The granulomas associated with *Mycobacterium tuberculosis* are composed of mononuclear (epithelioid) cells surrounded by lymphocytes, with or without Langhans-type multinucleated giant cells (Fig. 52.6). They are generally 1 to 2 mm in size, but large tuberculomas up to 12 cm have been reported (90). Caseation (central necrosis surrounded by peripheral macrophages) is considered a hallmark finding of TB granulomas, and is present in 33% to 100% of liver biopsy specimens from various series (86). Caseation is thought to occur as a result of overwhelming acute dissemination of mycobacterial organisms, and therefore,

occurs more commonly in miliary TB. It is characteristically granular and "cheesy" in appearance; hence the term "caseous" (Fig. 52.7A). Giant cells are often present and AFB stains are positive in approximately 60% (Fig. 52.7B), in contrast to cultures often being negative (87). PCR assays have been diagnostic in most patients with TB granulomas in the liver (91,92).



▪ **Figure 52.6** Granulomas with caseation necrosis in miliary tuberculosis (hematoxylin-eosin, ×100). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Clinical and biochemical clues to the presence of hepatic TB are nonspecific (86). Hyperglobulinemia is often seen and serum alkaline phosphatase levels are often raised, while aminotransferase values remain normal in pulmonary disease and mildly elevated in miliary TB (93,94). Jaundice is unusual and should prompt a search for biliary obstruction or associated hepatotoxicity from anti-TB drug therapy. An uncommon form of acute miliary TB described by Essop et al. (95), and called "tuberculous hepatitis" was accompanied by fever, tender hepatomegaly, and splenomegaly, and had a very high incidence (96%) of hepatic granulomas, with caseation seen in 83%. However, tubercle bacilli were found in only 9% of cases. The demonstration of AFB has generally been low in pulmonary TB, and higher in miliary TB; but positive cultures have been rare in the literature (86,96). Following successful anti-TB treatment, complete resolution of hepatic granulomas is described as occurring within a few months (95,97).



▪ **Figure 52.7 A:** Caseous necrosis with multinucleated Langhan giant cells in granulomas associated with *Mycobacterium tuberculosis* (hematoxylin-eosin, ×100). **B:** Higher magnification demonstrated acid-fast organisms (*arrows*) in a caseating granuloma (Ziehl-Neelsen, ×1000). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

TB affects 1% to 4% of liver allograft recipients and other solid organ transplantation patients (98). In a series of

42 postorthotopic liver transplantation patients with granulomas on liver biopsy, Ferrel et al. (99) found only 1 patient with documented TB for a prevalence of 2.4%. Granulomatous hepatitis has been attributed to isoniazid and pyrazinamide, although one wonders whether the underlying TB may have been responsible for the finding of granulomas in this setting. In general, hepatotoxicity from isoniazid and pyrazinamide takes the form of acute hepatocellular injury, including fulminant hepatic failure (86,100,101).

Rarely a mycobacterium other than *M. tuberculosis* is isolated from the liver. *M. kansasii*, *M. mucogenicum*, and other atypical mycobacterial organisms have been found (102).

Granulomatous hepatitis caused by *bacille Calmette-Guérin* (BCG) is reported in 12% to 28% of patients receiving BCG as immunotherapy for neoplastic disease (103,104), often several months after the last inoculation. A role for both Calmette-Guérin bacilli and a hypersensitivity reaction has been proposed as the mechanism. O'Brien and Hyslop (105) note that BCG organisms may remain viable for weeks to months. Hunt et al. (106) suggested that BCG preparations are antigenic, with granulomas forming as a result of a hypersensitivity response.

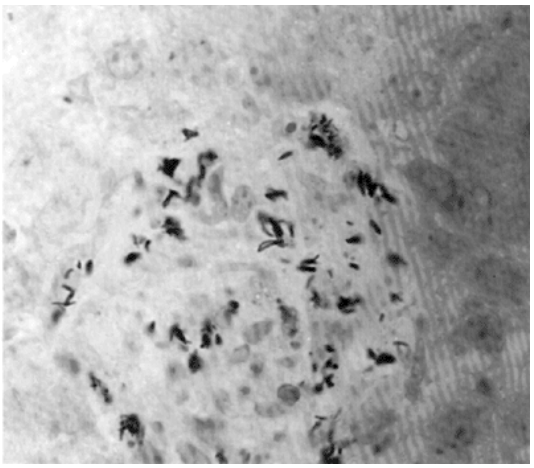
Leprosy (Mycobacterium Leprae)

Granulomas are common in lepromatous leprosy, but symptoms and signs of hepatic involvement are generally absent, apart from hyperglobulinemia (107,108). Histologically, a spectrum of epithelioid, tubercular, and foam-cell granulomas is seen (109); the first two suggesting the tuberculoid form of the disease and the latter lepromatous leprosy. Foam cells consist of Kupffer cells that are filled with *Mycobacterium leprae*. The *M. leprae* bacteria are much less common in epithelioid granulomas. Hepatic granulomas in leprosy correlated with cutaneous involvement, in the series by Chen et al. (108).

Granulomas in Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Hepatic granulomas were seen in 37% of 501 liver biopsies in one large series (110) and in 16% to 48% of smaller series (89,111,112). A microbiological diagnosis was apparent only after liver biopsy in more than 50% of a series of patients presenting with fever and elevated alkaline phosphatase and γ-glutamyltransferase levels (113). The most common cause of granulomas appears to be due to *Mycobacterium avium* complex (MAC) that has been present in 20% to 50% of all autopsy series in fatal AIDS cases and in 10% to 70% of those undergoing liver biopsy for suspected AIDS hepatopathy (89,114,115). MAC has been reported to develop in up to 20% of patients after an AIDS-defining illness has occurred. The granulomas are generally poorly formed and are composed of foamy histiocytes with a paucity of other cells, but they contain numerous AFB (Fig. 52.8). Granulomas may be absent in a severely immunosuppressed host. In a series of liver biopsies and autopsies from 71 patients with AIDS from 1982–1986 in Paris, Astagneau et al. (116) found granulomatous hepatitis in 22 patients, half of which were associated with opportunistic infections and the rest remained unexplained. Well-formed necrotizing and non-necrotizing granulomas are described in immunocompetent patients infected with MAC (117).

M. tuberculosis has been described in 60% of AIDS patients with pulmonary disease and in 7.5% with extrapulmonary disease, often as a result of reactivated disease from a previous focus (89,118). These granulomas tend to be better formed and occur earlier in the course of human immunodeficiency virus (HIV) infection compared to *M. avium* complex. However, fewer AFB are present on immunohistochemical stains. In contrast to *M. tuberculosis*, MAC is usually the result of a primary infection, typically in the late stages of AIDS with $CD4^+$ counts $<200/mm^3$ (89). An improved response to multidrug regimens has correlated with improved survival. Alkaline phosphatase values, reflecting hepatic involvement, may normalize after successful therapy.



▪ **Figure 52.8** *Mycobacterium avium* in a poorly formed granuloma in a patient with acquired immunodeficiency syndrome. Numerous acid-fast bacillus are seen in distended histiocytes (hematoxylin-eosin, ×630). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

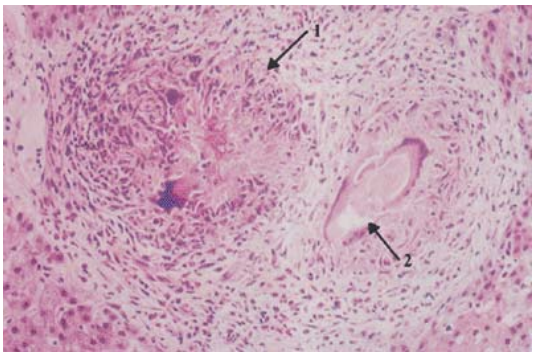
A number of parasites can cause granulomatous disease in the population with HIV. *Pneumocystis carinii* pneumonia with extrapulmonary spread is seen in more than 30% at autopsy. Toxoplasmosis, leishmaniasis, and schistosomiasis are described in this population. Fungal causes of granulomas may also be seen (58).

Other Infectious Causes of Granulomas

Schistosomiasis

Schistosomiasis (*Schistosoma mansoni, S. japonicum, S. mekongi*) is acquired by contact with fresh water infested with the parasitic larvae (cercariae) carried by their host snails. Several hundred million persons are affected worldwide, mostly in tropical regions of the Middle East, the Caribbean (Puerto Rico), Central America, Africa, and South East Asia. The life cycle of schistosomes is well described (35,119). The adult worms can survive in the host for up to 35 years, depositing hundreds to thousands of eggs daily. Most eggs find their way to the portal circulation and become trapped in several organs, notably the liver. Constant deposition of eggs results in a $CD4^+$ T-helper-cell mediated granulomatous response to egg antigens (Sm-p40 and others) (120,121,122,123,124), which can cause a chronic hepatic reaction with large granulomas in portal areas that become surrounded by fibrosis, leading to noncirrhotic

portal hypertension (125) (Fig. 52.9). Following the death of the ova, a dense portal fibrosis may be the only histologic remnant of the infection (18).



▪ **Figure 52.9** Schistosomiasis (*Schistosoma mansoni*) granuloma (*arrow 1*) next to an ovum blocking an intrahepatic portal vein branch (*arrow 2*) in a patient with portal hypertension. (hematoxylin-eosin, ×400). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Clinically, a serum sickness–like illness from circulating immune complexes produces fever, myalgias, arthralgias, cough, diarrhea (sometimes bloody), and eosinophilia. Eggs may be found in the stool, but an enzyme-linked immunosorbent assay (ELISA) test to detect schistosome antibodies is more reliable. Features of periportal fibrosis and portal hypertension on abdominal imaging tests may also be helpful in making the diagnosis. Friis et al. (126) described a quantitative serologic method to estimate the volume of *S. mansoni* granulomas in a murine model. They found that zinc-deficient animals had more eggs in their livers due to impaired intestinal expulsion and resorption of eggs. Jacobs et al. (127) noted that a *S. haematobium* infection modulated the *S. mansoni* egg antigen-induced granulomas and hepatic fibrosis in mice.

Q fever

This worldwide zoonosis among ruminants and birds is caused by tick-borne *Coxiella burnetti*, an obligate intracellular gram-negative bacterium forming spore-like forms that inhabits monocytes and macrophages (128,129). Acute infection in humans occurs primarily by inhalation of contaminated aerosols and dusts from domestic animals, particularly after contact with parturient females and their birth products as the placenta harbors millions of the bacteria (130). Q fever may present as a community-acquired pneumonia with high fever, cough, headache, and myalgias (131,132), or as a self-limited febrile illness without pulmonary symptoms (130). Up to 50% of patients present with acute hepatitis-like symptoms (131), several of whom have hyperbilirubinemia including jaundice (133). Hepatic involvement also presents as FUO and as an incidental finding in patients with pneumonia. Industrial outbreaks (from aerosolized spores) have been described (134), but the infection occurs most frequently among workers in sheep (55) and cattle farms (135) or in slaughterhouses (136) outside the United States. A chronic form of the disease can result in endocarditis, myocarditis, pericarditis, and chronic fatigue syndrome (137,138,139). Hepatitis generally affects younger patients, while pneumonia is seen in older individuals (54,139).

The characteristic histologic appearance is a fibrin-ring granuloma (47,140), previously referred to as a "doughnut" granulomas (141) (Fig. 52.3). They are usually seen in portal areas and accompanied by prominent lymphoid hyperplasia. Toll-like receptor 4 appears to play a role in the uptake of virulent organisms and the development of granulomas (128). In mice, the intraperitoneal route of infection led to hepatic involvement, while the intranasal route was more often associated with pneumonitis (142). Serology and PCR testing are available to confirm the diagnosis. Treatment has been successful with the use of doxycycline and steroids (54,139). A

preventative vaccine is available (136).

Syphilis

Secondary lues (*Treponema pallidum*) involves the liver in up to 50% of cases, often with tender hepatomegaly and, sometimes, overt jaundice. Elevated alkaline phosphatase levels with lesser elevations in aminotransferases is the most common biochemical finding. Spirochetes have been demonstrated by appropriate staining in up to half of affected liver specimens (143). Following successful antibiotic treatment, resolution of granulomas and other hepatic abnormalities can be expected. In tertiary syphilis, single or multiple hepatic gummas are seen, that may give rise to a lobulated appearance (hepar lobatum). Although much larger than granulomas, gummas may also resolve following treatment (35).

Yersinia enterocolitica

Diffuse granulomas of the liver and spleen are seen in the septicemic form. Twelve percent of infections have involved the liver, in some cases causing chronic hepatitis (144,145).

Bartonella henselae (catscratch fever)

Catscratch fever develops after a scratch or bite by a cat or kitten and usually results in regional lymphadenitis, although, peripheral adenopathy is not always present (146,147,148). Scattered granulomas with central necrosis may coalesce to form abscesses. This disease has been

diagnosed in a liver transplant recipient with a FUO (149).

Warthin-Starry silver staining may identify the gram-negative bacillus early in the course of infection. An antigen skin test is positive in 90% but PCR tests are now available (150).

Visceral leishmaniasis

Visceral leishmaniasis (*Leishmania donovani*, kala azar) is transmitted by the bite of the sandfly causing a papular or ulcerative skin lesion with systemic symptoms occurring after a latent period of 2 to 6 months or longer. Intermittent fever, weight loss, tender hepatosplenomegaly, and occasional jaundice are seen. Phagocytosis of the parasite by macrophages in the reticuloendothelial system (RES) allows the organism to multiply in the affected liver, spleen, bone marrow, and lymph nodes. The diagnosis is based on the finding of amastigotes in aspirates or promastigotes in culture from these tissues, as well as from serologic testing (51,151).

Visceral larva migrans

Visceral larva migrans caused by *Toxocara canis*, typically produces hepatic granulomas containing numerous eosinophils. In a series of 43 cases reviewed by Epstein et al. from the AFIP (152), 30% were younger than 20 years and 60% were asymptomatic with the disease discovered incidentally. Fever and/or abdominal pain were the most common symptoms reported in the remainder. Granulomas were multiple in 61% and central necrosis was characteristically surrounded by eosinophils and neutrophils, often with Charcot-Leyden crystals. Remnants of the parasite were detected in 23%, and immunohistochemical staining and serology confirmed the diagnosis in many others.

Brucellosis

Acquired from infected cattle (*Brucella abortus*), pigs (*B. suis*), sheep (*B. ovis*), and goats (*B. melitensis*), often from raw, unpasteurized milk and dairy products, brucellosis typically presents as an acute febrile illness with hepatic involvement.(153,154,155). Jaundice is not uncommon (155,156), as is relapsing (undulant) fever. Epithelioid granulomas are most often seen in the chronic form of the disease, with a lobular location more common that the portal tracts. Central necrosis, a polymorphic infiltrate, relatively few giant cells, and peripheral fibrosis are typically seen. The diagnosis is confirmed by serology, positive Brucella PCR (153,154), or culture of liver tissue (153). Untreated, the clinical course is usually one of spontaneous resolution within 3 to 12 months of infection, although antibiotics may shorten the illness (35,155).

Histoplasmosis

Histoplasmosis (*Histoplasma capsulatum*) is found most commonly in the midwest and central parts of the United States, especially river valleys, and usually affects young children more than adults (19). Four percent to 8.5% of patients have rare well-formed granulomas (19,157). Rarely are they caseating, resembling TB. Clinically, adrenal insufficiency may be a diagnostic clue (157).

Coccidioidomycosis

Coccidioidomycosis (*Coccidioides immitus*) is most likely to be found in the deserts of the southwest United States. Granulomas are more likely to be found in the liver compared to histoplasmosis, and eosinophilia is often present (158).

Granulomas Associated with Chronic Viral Hepatitis and its Therapy

Several reports have described granulomas in patients with *hepatitis C* infection (159,160,161,162,163), although some of these may be related to suspected or unsuspected sarcoidosis (78,164) or PBC (165). Emile et al. (160) described multiple epithelioid granulomas in 10% of cirrhotic livers without any other cause being identified. These granulomas were located within cirrhotic nodules and not in portal areas.

Interferon has been associated with granuloma formation in patients with hepatitis C virus (HCV) infection (159,160,166) although the exact pathogenesis is not always certain. Interferon-induced cutaneous sarcoid has been described in patients with hepatitis C (80), and induction (81) and reactivation (79) of systemic sarcoidosis has also occurred, possibly through the production of inflammatory cytokines mediated by interferon (167).

Granulomas are also found in a small percentage of patients with chronic hepatitis B, 1.5% among 663 patients in one series (168).

Granulomas *after liver transplantation* are reported to occur in 3% to 9% of allografts (99) and may reflect recurrence of the original disorder, such as PBC, sarcoidosis, and so on, or possibly de novo formation from interferon therapy for HCV in the post-transplantation setting. Granulomatous cholangitis may be seen in acute cellular rejection or a delayed vanishing bile duct syndrome (VBDS) (169). Infections with opportunistic agents, including TB (86) and *Toxoplasma gondii* are described. Toxoplasmosis appears within the first 3 months with fever, pneumonia, and possibly meningitis or encephalitis, usually from activation of a latent infection (170) (Table 52.8).

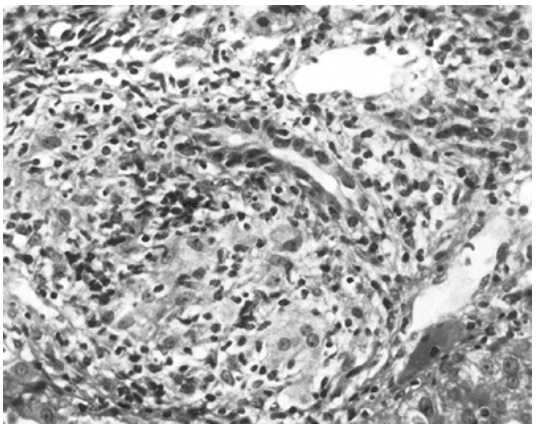
Table 52.8. Causes of Granulomas in the Post–Liver Transplantation Setting

Recurrence of primary disease (primary biliary cirrhosis, sarcoidosis, hepatitis C virus, hepatitis C virus)
Interferon therapy–induced
Acute cellular rejection (granulomatous cholangitis)
Chronic ductopenic rejection (vanishing bile duct syndrome)
Post-transplantation opportunistic infections (cytomegalovirus, tuberculosis, fungi, toxoplasmosis)

Granulomas in Other Chronic Cholestatic Liver Diseases

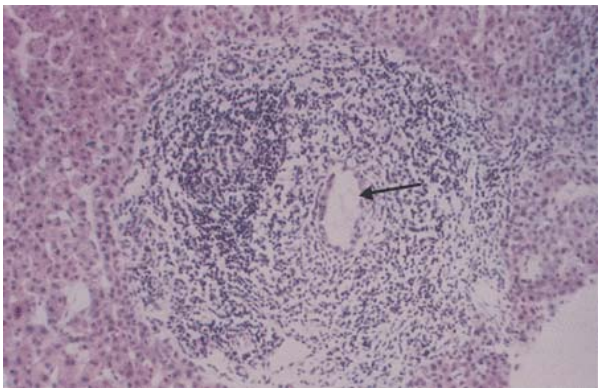
Primary biliary cirrhosis

Epithelioid granulomas are found in at least 25% of patients with PBC, usually in the early stages of the disease (171). PBC typically affects middle-aged women who may present with subclinical alkaline phosphatase elevations or with symptoms reflecting chronic cholestasis, including pruritus. The disease is associated with positive antimitochonrial antibodies (AMA) in >95% and this may serve to differentiate PBC from sarcoidosis. In addition, PBC often occurs in association with other autoimmune disorders, such as the sicca syndrome, Sjögrens syndrome, and CREST syndrome among others (171). Granulomas in the portal areas are in close proximity to injured bile ducts, and may give the appearance of being germinal centers (Figs. 52.10 and 52.11). It is thought that they form as part of the immune-mediated ductal injury, but release of bile acids and phospholipids from injured ducts may also contribute to their development (172,173). Granulomas located in the hepatic lobules are noncaseating and show less central matrix deposition than is usually seen in sarcoidosis (73). As PBC progresses, granulomas become less frequent (174). However, treatment with ursodiol therapy may also be associated with a reduction in granulomas, through its beneficial effects on the histologic features of cholestasis (175,176). The presence of granulomatous bile duct lesions can help differentiate and confirm the diagnosis of recurrent PBC from chronic rejection after liver transplantation (174).



▪ **Figure 52.10** Granulomatous cholangitis in primary biliary cirrhosis. (hematoxylin-eosin, ×400). (Courtesy

of Dr. KG Ishak, Armed Forces Institute of Pathology.)



▪ **Figure 52.11** PBC with a granuloma in an expanded portal area near a damaged bile duct (arrow) (hematoxylin-eosin, ×160). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Primary sclerosing cholangitis

Granulomas have also been reported in PSC (172,177). Thirteen percent of 100 patients undergoing liver transplantation for PSC had noncaseating, non-necrotizing epithelioid granulomas, some with giant cells (177). They were found in the portal areas, scars, and the hepatic parenchyma, and were present in all stages of the disease, but do not represent granulomatous cholangitis (in contrast to PBC where the granulomas are a feature of duct destruction). Ludwig et al. (177) surmise that the granulomas in PSC form in response to bile acid leakage. Perigranulomatous lymphocytic infiltrates were common.

Drug-Induced Granulomas

More than 60 drugs have been implicated (Table 52.9), although, most are isolated case reports (8,67,100,178). A few agents appear to be well-documented as causing granulomatous hepatitis, usually as part of a hypersensitivity syndrome (e.g., phenylbutazone [PBZ]) (179) (Fig. 52.12A,B), sulfonamides, allopurinol (48) (Fig. 52.3B), phenothiazines, and penicillins. Drug-induced granulomas have been reported with a frequency of up to 29% of all causes of hepatic granulomas (8); although the prevalence has usually been far lower. In the McMaster series (8), the drugs that were listed as probably or possibly related were predominantly from antihypertensive, antirheumatic, anticonvulsant, and

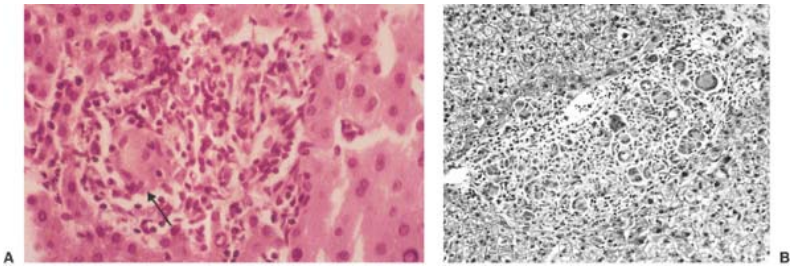
antimicrobial drug classes, and included methyldopa, hydralazine, phenytoin, isoniazid, cephalexin, penicillin, sulfonamides, and procainamide, among others. Six percent to 9.5% of granulomas in other series were due to many of the same drug classes (10,11,16) and included chlorpropamide, allopurinol, PBZ, synthetic penicillins, quinidine, carbamazepine, and phenothiazines, among others.

Table 52.9. Drugs Associated with Granulomas and Granulomatous Reactions

Allopurinol	Nitrofurantoin
Amiodarone	Norfloxacin
Amoxicillin—clavulanic acid	Oral contraceptives
Aprindine	Oxacillin
Carbamazepine	Oxyphenbutazone
Chlorpromazine	Papaverine
Chlorpropamide	Penicillin

Dapsone	Phenprocoumon
Diazepam	Phenbutazone
Dicloxacillin	Phenytoin
Diltiazem	Procainamide
Disopyramide	Procarbazine
Glyburide	Pronestyl
Glibenclamide	Pyrazinamide
Gold salts	Pyrimethamine-chloroquine
Green juice	Quinidine
Halothane	Quinine
Hydralazine	Rantidine
Interferon α	Rosiglitazone
Isoniazid	Sulfasalazine
Mebendazole	Saridon
Mesalamine	Seatone (green lipped mussel extract)
Methimazole	Sulfanilamide and other sulfa drugs
Methotrexate	Tetrabamate (Atrium)
Methyl dopa	Tetrahydroaminoacridine (tacrine)
Mineral oil	Tolbutamide
References contained in 57,58,67,100,101,178.	

The most frequent clinical presentation is an acute febrile illness, with or without a rash and peripheral eosinophilia, followed by jaundice and biochemical evidence of hepatic dysfunction (8). A latency of 1 to 16 weeks is described by McMaster et al. (8), which is consistent with the timeframe for other hypersensitivity reactions from drugs (100,101). Histologic features suggesting a drug-induced cause include the relatively uniform age of the granulomas, and the presence of eosinophils, apoptotic bodies, acute cholangitis, and/or vasculitis (67). In contrast, tissue eosinophilia is not seen in sarcoidosis or TB. The prognosis is generally good with recovery after the offending agent is withdrawn. Healing without sequellae is the rule (8,67).



▪ **Figure 52.12** Granulomas associated with acute hypersensitivity reaction to phenylbutazone. **A:** Two epithelioid granulomas similar in size and age (hematoxylin-eosin, ×100). **B:** Epithelioid granuloma containing several multinucleated giant cells (hematoxylin-eosin, ×150). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

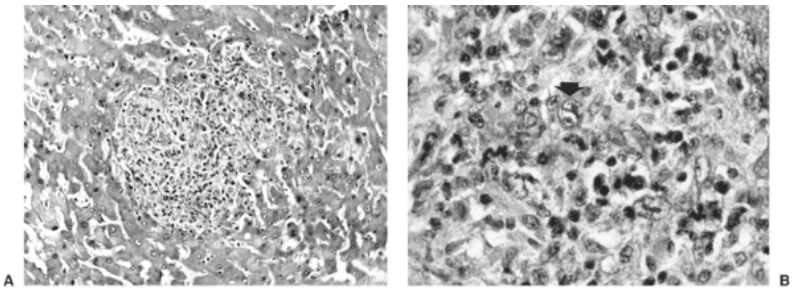
Granulomas Associated with Metals and Minerals

Beryllium poisoning uncommonly involves the liver, as the chronic form of the disease is primarily a pulmonary granulomatosis with dyspnea and cough (180,181). It should be suspected in occupational exposure where beryllium is employed, such as in the manufacture of ceramics and alloys, and in atomic energy workers. The granulomas seen in beryllium disease may contain Schaumann and asteroid inclusion bodies, which are the end products of actively secreting epithelioid cells (3) and may be confused with those seen in sarcoidosis (182). Tissue analysis for beryllium may be confirmatory.

Talc microcrystals are commonly seen in intravenous drug users, many of whom "cut" their heroin with talcum powder. They are found in hypertrophied portal macrophages, but generally do not develop into well-formed granulomas (183), in contrast to *silica*, which forms foreign body–type giant cells that contain the birefringent crystals (184). The presence of talc in the liver, however, may be an important clue as to the etiology of chronic hepatitis C (185).

Refractile *silicone* particles (confirmed by x-ray energy dispersive spectroscopy) were described in association with sarcoid-like granulomas, some with giant cells, in hemodialysis patients in whom embolization (spallation) of silicone was traced back to tubing traumatized in the roller pump (186,187). Abnormalities in liver-associated enzymes persisted more than 4 years later in two patients (188).

Multiple granulomas were described in long-term hemodialysis patients (189) with *aluminum* found in the cytoplasm of macrophages in the liver, spleen, and lymph nodes.



▪ **Figure 52.13** Hodgkin's disease granulomas. **A:** Focal granulomatous infiltrate (hematoxylin-eosin, ×180). **B:** Higher magnification showing a pleomorphic infiltrate with a Reed-Sternberg cell (*arrow*) (hematoxylin-eosin, ×750). (Courtesy of Dr. KG Ishak, Armed Forces Institute of Pathology.)

Granulomas Associated with Malignancy and Immunodeficiency States

HD, lymphomas, and certain hematogenous malignancies (e.g., *hairy cell leukemia* [HCL] are associated with granulomas. Granulomas are seen in approximately 10% of those with HD (190,191) (Fig. 52.13A, B), and were present in 31% of those with HCL (192). Granulomas in the liver may precede the diagnosis of HD or lymphoma (193), and may be a source of diagnostic confusion with sarcoidosis (3). Rarely have the granulomas associated with HD been caseating (194). The presence of granulomas does not appear to convey any clinical advantage as

suggested by early reports (195). Mimics of lymphomas that may be associated with hepatic granulomas include *Kikuchi lymphadenitis* among others (196).

Common variable immunodeficiency is associated with noncaseating granulomas in the liver and spleen (197). *Chronic granulomatous disease* (198) may appear in adults as well as children (199). Hypogammaglobulinemia has been associated with granulomas and asymptomatic cholestasis (200).

Miscellaneous Causes of Granulomas

Jejunoileal bypass–granulomas were among several hepatic lesions, including steatohepatitis, which were seen following jejunoileal bypass surgery for morbid obesity (201). Twenty-four percent of patients undergoing jejunoileal bypass in a series of 25 patients reported by Banner et al. developed granulomas (202). They developed within 3 months to 4 years of the

surgery and were much more common than the incidence (4%) of granulomas seen in obese patients before surgery. Their exact etiopathogenesis is unclear.

Table 52.10. Hepatic Granulomas as a Cause of Fever of Unknown Origin

Author (ref)	Year	Number of cases	Diagnoses
Terplan (205)	1971	*n* = 60	Sarcoidosis 47%; TB 25%; idiopathic 17%
Bruguera (206)	1981	*n* = 25	36% "positive" diagnosis
Israel (204)	1984	*n* = 15	Sarcoidosis 100%
Telenti (26)	1989	*n* = 20	Idiopathic 100%
Zoutman (27)	1991	*n* = 23	Idiopathic 74% (41% spontaneous regression); 26% specific diagnosis (Q fever, TB, histoplasmosis)

TB, tuberculosis.

Rheumatoid arthritis is a rare cause of granulomas (203), as are other rheumatic disorders.

Idiopathic granulomatous hepatitis and fever of unknown origin–up to 74% of hepatic granulomas in the early series were not identified with a specific diagnosis (27) (Table 52.10). Many of these patients had FUO or other systemic symptoms or hypersensitivity features, and were designated as having "granulomatous hepatitis" (25). Sarcoidosis was found in a number of such patients (204,205); and infectious agents in some of the others (205,206); although no cause remained apparent in many (26), Aderka et al. (207) found that certain clinical parameters could differentiate idiopathic granulomas from those that are secondary to lymphoma and other malignancies—namely smaller spleen size, small liver size (<4 cm below the respective costal margin), lower percentage of eosinophils (<4%), and fever lasting <4 weeks. The spectrum of hepatic candidiasis includes granulomas, especially among patients with hematological malignancies presenting with fever, and elevated alkaline phosphatase levels (208). In a series by Cunningham et al. (9), 31% of granulomas that were considered initially to be "idiopathic", were subsequently diagnosed as having a specific cause based on a more in-depth study. As a result, fewer than 10% remained without an etiology (9), on par with later series. Schlegel (60) described what he termed "factitious" granulomatous hepatitis in a health care worker who had hepatic granulomas and leukopenia attributed to quinine ingestion that was likely being taken surreptitiously.

Treatment of Hepatic Granulomas and Granulomatous Hepatitis: General Principles

Simple granulomas found incidentally are often asymptomatic but should prompt a workup for the most common causes of granulomas (Tables 52.3, 52.5, 52.9). They may not require treatment if involvement is isolated to the liver. Individuals presenting with granulomatous hepatitis, including those with FUO and other systemic symptoms, can be treated for a specific cause, if found. Those granulomas that are considered *idiopathic* may respond to an empiric course of corticosteroids (5,25,26,27). In a series of 23 cases reported by Zoutman et al. (27) presenting with FUO, 74% were considered idiopathic. In 41% of this group the granulomas resolved spontaneously, while the remaining 59% received corticosteroids or indomethacin; 18% were short-term and 41% long-term treatment (mean 33 months). All were described as having remained afebrile and healthy after a 5-year follow-up. A similarly good prognosis after long-term corticosteroid therapy of up to 10 years was described by Telenti and Hermans (26) with no progression or dissemination of an unrecognized infectious process.

In sarcoidosis, corticosteroids are usually given when there is evidence of extrahepatic sarcoidosis, as most

experts do not believe that isolated hepatic involvement requires therapy unless there is evidence of severe cholestasis (68). Methotrexate has also been utilized in this disorder (209), as has ursodiol (68), especially for the cholestatic form. Liver transplantation has been required to treat some patients with end-stage chronic cholestatic sarcoidosis (76).

Specific antibiotic therapy for various infectious causes and other treatment options directed at specific causes of hepatic granulomas are reviewed elsewhere (35) (Table 52.5). Empiric treatment for TB has been offered in some instances (209). Drug-induced causes generally resolve spontaneously once the medication has been discontinued.

In cases of symptomatic idiopathic granulomatous hepatitis (such as FUO), corticosteroids have been the mainstay of treatment (26). Longstreth and Bender (210) described the use of cyclophosphamide as a steroid-sparing maintenance agent in preventing recurrence of idiopathic hepatic granulomatosis. Methotrexate was described as being effective in seven patients presenting with fever and anorexia in a series by Knox et al. (209). Nonsteroidal anti-inflammatory

drugs (NSAIDs) were used successfully in a child with necrotizing granulomatous inflammation (211).

Summary

Hepatic granulomas are not infrequently encountered in the evaluation of a variety of infectious, chronic, cholestatic, inflammatory, hypersensitivity-mediated, and neoplastic disorders. Granulomas can be expected to be present in 5% to 10% of routine liver biopsies, and in a substantially higher percentage of patients with suspected localized or systemic granulomatous diseases. Both genders and all ages can be affected. The list of possible etiologies, including dozens of drug-related causes, has been expanding, although certain causes still predominate, namely sarcoidosis, TB, PBC, and certain drugs (especially those acting through hypersensitivity mechanisms), often relating to the geographic location of the patient. Less common causes remain a diagnostic challenge, but are being found more frequently, commensurate with the wider availability of serologic and other PCR-based test systems. Hepatic granulomas may be the only histologic clue as to the presence of several infections, such as TB and Q fever, among others. Once found, a decision regarding the extent of a further diagnostic evaluation must be made, and ultimately, what treatment, if any, is required. The cause of approximately 10% of granulomas remains undefined (idiopathic), some of which are associated with unexplained fever and other symptoms (including arthralgias and rash), that define "granulomatous hepatitis." These cases are arguably the most challenging, often having to rely on empirical treatment regimens. It is anticipated that advances in clinical and histopathologic diagnostic methodologies will help identify even the most refractory causes of hepatic granulomas in the future.

Annotated References

Collins MH, Jiang B, Croffie JM, et al. Hepatic granulomas in children. A clinicopathologic analysis of 23 cases including polymerase chain reaction for histoplasma. *Am J Surg Pathol* 1996;20:332–338.

One of the largest reviews of granulomas in a pediatric population. In comparison to adults where the most common causes are sarcoidosis, TB, drugs, neoplasms, and chronic cholestatic liver diseases, in this series, histoplasmosis accounted for 65% of granulomas with an identifiable etiology. The authors emphasize the usefulness of PCR-based testing to improve diagnostic accuracy.

Devaney K, Goodman ZD, Epstein MS, et al. Hepatic sarcoidosis. Clinicopathologic features of 100 patients. *Am J Surg Pahtol* 1993;17:1272–1280.

One of the largest series of hepatic granulomas due to sarcoidosis from the experts at the Armed Forces Institute of Pathology. They describe three main categories of hepatic sarcoidosis based on biochemical and histologic features: Cholestatic in 58% (often with bile duct injury and ductopenia similar to PBC), necroinflammatory in 41%, and those with associated vascular changes of sinusoidal dilatation or nodular regenerative hyperplasia in 20%.

Ishak KG, Zimmerman HJ. Drug-induced and toxic granulomatous hepatitis. *Baillieres Clin Gastroenterol* 1988;2:463–480.

The most comprehensive review of drugs and chemical toxins associated with hepatic granulomas from two of the leading experts in the fields of drug-induced hepatotoxicity and hepatopathology. They emphasized the role of liver biopsy in this setting. Histologic lesions that suggested a drug etiology included associated tissue eosinophilia, acute cholangitis, and the uniform age of the granulomas. Special stains and other histopathologic analyses may also play an important role in helping to confirm the diagnosis and assigning causality to a drug.

James DG. A clinicopathological classification of granulomatous disorders. *Postgrad Med J* 2000;76:457–465.

An excellent review of the pathophysiology and immunology of granuloma formation. The author describes the interplay of the invading organism, drug, chemical, or other irritants, and the cytokines and other biological mediators involved in the transformation of macrophages to epithelioid cells that comprise a majority of granulomas. An overview of many infectious, chemical, and other causes of granulomas is provided.

Lewis JH, Zimmerman HJ. Tuberculosis of the liver and biliary tract. In: Schlossberg D, ed. Tuberculosis and

nontuberculous mycobacterial infections, 4th ed. Philadelphia: WB Saunders, 1999:238–263.

A comprehensive review of tuberculous infections of the liver and the spectrum of granulomatous disease they produce. Hepatic granulomas are found most often in miliary TB and less often in pulmonary and extrapulmonary disease. Caseating necrosis is seen most commonly in association with the acute disseminating infections of miliary TB. The yield of special stains and culture remains low, but PCR-based tests of TB have improved disgnostic yields.

Raoult D, Tissot-Dupont H, Foncault C, et al. Q fever 1985–1998. Clinical and epidemiologic features of 1,383 infections. *Medicine (Baltimore)* 2000;79:109–123.

A comprehensive review of Q fever from France. This organism (Coxiella burnetti) *is responsible for a characteristic fibrin-ring type of granuloma. Acute hepatitis was the most common clinical presentation, seen in 40% of cases, often associated with Q fever pneumonitis. The authors describe a number of patient host factors that appear to dictate the clinical expressions of the disease.*

Wanless IR, Geddie WR. Mineral oil lipogranulomata in liver and spleen. A study of 465 autopsies. *Arch Pathol Lab Med* 1985;109:283–286.

Lipogranulomas are less common than epithelioid granulomas, but were found in 48% of livers examined in this large series. The authors describe the clinicohistologic features of lipogranulomas that are generally clinically silent. They appear most often in older adults, particularly men, and are associated with the ingestion of mineral oil in most instances.

Zoutman DE, Ralph ED, Frei JV. Granulomatous hepatitis and fever of unknown origin. An 11-year experience of 23 cases with three years' follow-up. *J Clin Gastroenterol* 1993;17:69–75.

One of the largest series that helps define the natural history of idiopathic granulomatous hepatitis associated with FUO. The authors were able to identify a specific diagnosis in only 26% of their patients. Among the cases without a precise etiology, 41% eventually resolved spontaneously; 18% resolved after short-term treatment with corticosteroids and anti-inflammatory drugs while the remaining 41% required long-term corticosteroid therapy to maintain clinical remission and prevention of fever over nearly 6 years of follow-up.

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Chapter 53

Selection and Timing of Liver Transplantation

Richard B. Freeman Jr.

Key Concepts

- Selection of candidates for liver transplantation is not necessarily linked to the timing of liver transplantation due to the severely constrained organ donor resource. This means that individual candidates cannot always receive a transplant at the optimal time for his or her benefit.
- For most patients with chronic liver disease, selection for transplantation depends on their risk of dying from the liver disease weighed against their risk of dying from the transplantation procedure and attendant medications. Mortality risk scores have been helpful in determining when this risk of death without transplantation is greater than the risk after the procedure. Although patients with severe chronic liver disease have deteriorated health-related quality of life (HRQOL), this must be weighed carefully against the risk of death while waiting compared with the risk of death from the transplantation. There can be no quality of life for deceased candidates.
- For some liver conditions, the benefit of liver transplantation cannot be weighed against the mortality risk from intrinsic liver disease. Therefore other methods, such as estimates of disease progression, must be used for proper selection of waiting candidates. Disease progression estimates are limited by lack of good natural history data.
- Pediatric patients with liver disease face unique problems and mortality risk models utilizing variables specific for children have been developed to address these differences.
- Efficient selection of liver transplantation candidates with acute liver failure is especially challenging because of the need to rapidly assess the probable natural course of the disease so that patients not likely to recover will receive transplant priority but those more likely to recover do not receive needless transplants.
- Living donor liver transplantation improves the ability of the clinician to "time" the transplantation at the most advantageous point in disease progression for the waiting candidate. However, donor risks and informed consent must not be subjugated in an effort to maximize the timing benefit.

Inhabitants in the developed world are fortunate that, in large measure, effective treatments for most medical conditions are generally available with access to these treatments limited mostly by socioeconomic or distributive problems, rather

than scarcity of the therapeutic substrate itself. This is not the case for transplantation. Unlike any other field in medicine, the clinician's ability to apply transplantation therapy is not completely defined by the risk-benefit ratio of that therapy for the patient in question. For patients who could potentially benefit from liver transplantation, the constrained resource problem is acutely severe because alternative liver support technologies are not perfected. Therefore, the application of liver

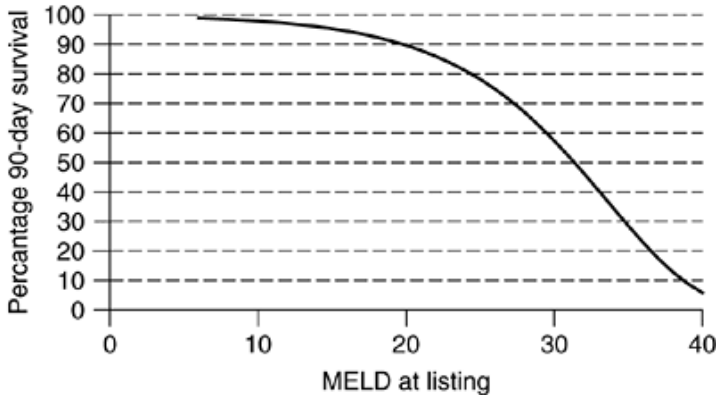
transplantation therapy, depends not only on the diagnosis of a problem for which liver transplantation is likely to provide more benefit than harm, but also on the availability of the therapeutic substrate; the liver itself. Consequently, and in contrast to most other medical conditions, determining if a patient diagnosed with progressive liver disease will receive benefit from liver transplantation is not sufficient for initiation of delivery of the treatment. This makes the optimal timing for liver transplantation a two-sided proposition: (a) Determining when a patient's disease has advanced far enough that he or she will receive more benefit than harm from the transplantation procedure and subsequent maintenance treatments, and (b) determining who, among all those who are deemed to likely receive benefit, should come first when donor constraints allow only one patient at a time to be treated. The former patient-based issue can be categorized as selection of appropriate candidates for transplantation. The second topic, timing of liver transplantation, is not entirely under the control of the treating physician or patient but much more influenced by allocation and distribution rules. Only in the case of living liver donor transplantation, can treating clinicians completely control the timing of liver transplantation for candidates they have deemed appropriate. In this chapter, the selection of patients for liver transplantation, the optimal timing of liver transplantation, absent organ donor constraints, the timing of liver transplantation in the context of living donor liver transplantation, and finally organ allocation priorities and their influence on the timing of liver transplantation are addressed.

Selection of Candidates for Liver Transplantation

Mortality Risk

Liver transplantation, like all therapies, should be offered when the risks are outweighed by the benefits. Quantitating risk and benefit however, is no easy proposition, and fraught with subjective interpretations of need for transplantation. For any candidate, comorbid conditions, such as cardiopulmonary disease (1), renal function, chronic or active acute infections, neurologic and psychiatric impairments as well as the social support system available to the patient must be assessed and considered in the overall determination of surgical risk. Caregivers and patients must weigh mortality risks, disease progression, and the impact of deterioration in quality of life for patients with liver disease who may be candidates for liver transplantation. In the past, most patients were deemed reasonable candidates for transplantation based on the development of signs or symptoms of decompensation usually related to portal hypertension (2). These original assessments of liver transplantation candidacy were subsequently incorporated into minimal listing criteria based on an anticipated 1-year survival of 90% or less if no transplantation was performed (3). This 1-year waiting list survival criterion was equated to a Child-Turcotte-Pugh (CTP) (4) score of greater than or equal to 7. However, the use of subjective variables, and differences in cholestatic versus noncholestatic liver diseases, as well as a “ceiling effect”

inherent in the CTP score for more advanced chronic liver disease, led investigators to develop mathematical models for primary biliary cirrhosis (5,6,7,8,9), and primary sclerosing cholangitis (10) to predict mortality. Subsequently, these models were compared to the CTP score and found to have similar accuracy for prediction of mortality risk (11). More recently, Malinchoc et al. defined a mathematical model to predict mortality risk in any patient with portal hypertensive complications undergoing transjugular intrahepatic portosystemic shunt (TIPS) procedure regardless of the underlying chronic liver disease (12) and without using the subjective variables employed by the CTP score (Table 53.1). This so-called Model for End-Stage Liver Disease (MELD) score has subsequently been shown to be highly predictive of 3-month mortality for a variety of cohorts of patients with chronic liver disease (13,14) and for a cohort of US patients waiting for liver transplantation (15). The MELD model has been widely accepted as a measure of chronic liver disease severity where severity of disease is defined as 3-month mortality risk and has been adapted to be incorporated into US liver allocation policy for determination of priority on the waiting list (16) (Fig. 53.1 and Table 53.1). Therefore, if one defines the need for liver transplantation in terms of risk of dying of liver disease, the MELD score provides an objective, readily available, easily applied, measure for selection of liver transplantation candidates with chronic liver disease.



▪ **Figure 53.1** Plot of Model for End-Stage Liver Disease (MELD) score at listing versus survival fraction for new listings on the liver transplantation waiting list 15 September, 2001, to 15 February, 2002.

Table 53.1. Model for End-Stage Liver Disease Score and Pediatric End-Stage Liver Disease Score

A. Malinchoc et al. (12)
$R = 0.957 \times \log_e(\text{creatinine mg/dL}) + 0.378 \times \log_e(\text{bilirubin mg/dL}) + 1.120 \times \log_e(\text{INR}) + 0.643 \times (\text{disease etiology}^a)$
B. MELD score UNOS/OPTN Policy
$R = (0.957 \times \log_e(\text{creatinine mg/dL}) + 0.378 \times \log_e(\text{total bilirubin}$

mg/dL) + 1.120 × $\log_e$(INR) + 0.643) × 10

PELD score

R = (0.463 (age[b]) – 0.687 × $\log_e$(albumin g/dL) + 0.480 × $\log_e$(total bilirubin mg/dL) + 1.857 × $\log_e$(INR) + 0.667 (growth failure[c])) × 10

MELD score as originally reported by Malinchoc et al. (12) (A), and as modified for US organ allocation (B). PELD score as reported by McDairmid et al. (17) and as is currently used for US pediatric liver allocation policy.

[a]1 for noncholestatic disease, 0 for cholestatic disease.
[b]<1 year of age + 1, ≥1 year of age = 0.
[c]>2 standard deviations below the mean for age = 1, ≤2 standard deviations below the median for age = 0.
MELD, Model for End-Stage Liver Disease; INR, international normalized ratio; UNOS, United Network Organ Sharing; OPTN, Organ Procurement and Transplantation Network; PELD, pediatric end-stage liver disease.

Health-Related Quality of Life

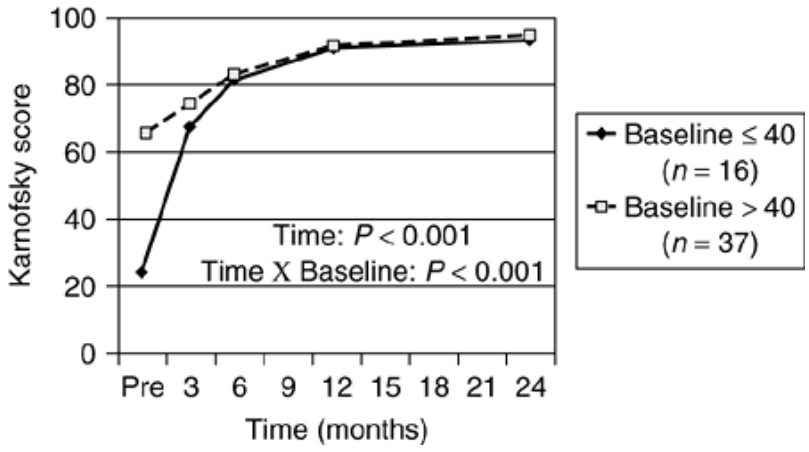
Mortality risk, however, may not be the only measure of need for liver transplantation. Numerous studies have documented that health-related quality of life (HRQOL) is poor for patients with progressive chronic liver disease (18,19,20) and that measures of mortality risk may not necessarily correlate with measures of HRQOL for these patients (21). Nonetheless, when weighing quality-of-life considerations physicians must account for mortality risk because, for patients who die, there can be no *quality* of life. Therefore, mortality risk usually takes precedence in determining the appropriate timing of liver transplantation for individual patients when the risks of death from transplantation surgery and immunosuppression are relatively low in relation to the mortality risk without transplantation although the patient's quality of life may be poor (22). For example, offering liver transplantation to patients with limitations in their HRQOL, who face a higher risk of dying because of the surgery and post-transplantation treatments than they will if they wait until their disease becomes more severe, may not be in their best interest. However, in one study of patients with end-stage liver disease, the subjects were frequently willing to accept a reduction in life expectancy in return for improved health and one half of the subjects accepted a 50% mortality risk in exchange for perfect health (23). Interestingly, almost all studies have shown significant improvements in HRQOL after transplantation (24), and the HRQOL outcome is not associated with the severity of illness before transplantation (25,26,27). Therefore, regardless of how ill patients are before the transplantation, if they survive, most can expect a reasonable HRQOL or functional status (Fig. 53.2). Therefore, there does not seem to be a justification for selecting one group of patients for liver transplantation because they are likely to achieve a better HRQOL afterwards than another group of patients. The available literature suggests that all recipients report relatively similar and significantly improved HRQOL afterwards.

Transplantation Benefit

The concept of selecting patients on the basis of estimates of transplantation benefit, as measured in number of life-years gained, has been recently addressed. Merion et al. compared the mortality risk for liver transplantation candidates remaining on the list with mortality risk for recipients of deceased donor liver transplants (DDLTs) and stratified their results by MELD score on listing. These investigators found that recipients with MELD scores less than 15 experienced a higher hazard for death than the candidates who remained on the list without transplantation at 1 year of follow-up. The authors concluded that, "liver transplants are being performed for some candidates who have a higher risk of dying from the transplantation procedure than they have from dying from their underlying liver disease" (29) (Fig. 53.3).

P.1456

It is interesting to note that in this study there was no MELD score beyond which a benefit in survival was obtainable. Therefore, even for candidates with very high pretransplantation mortality risks, as defined by their MELD scores, a significant benefit is achieved by liver transplantation because the success rate is still acceptable and their survival probability without transplantation is essentially zero. However, the post-transplantation survival results are based only on the candidates for whom liver transplantation was performed, and many more patients with high MELD scores than those with lower MELD scores are removed from the waiting list for reasons of death or being too sick (30). These results indicate that there is a selection process by which centers are choosing candidates with high MELD scores for whom they expect a good chance of success. From these data, the optimal timing of liver transplantation for patients with chronic liver disease can be estimated on the basis of their mortality risk, as defined by their MELD score. Most patients with nonmalignant liver disease, who have MELD scores greater than 15, will receive a benefit from liver transplantation, whereas those with lower MELD scores have a better chance of surviving for 1 year without transplantation.



▪ **Figure 53.2** Karnofsky performance scores for patients before and at various times after liver transplantation. Patients with poor baseline scores (*solid line*) had similar performance scores after transplantation compared with patients with less severe performance scores (*dotted line*) (28). (From Pinson CW, Feurer ID, Payne JL, et al. Health-related quality of life after different types of solid organ transplantation. *Ann Surg* 2000;232(4):597–

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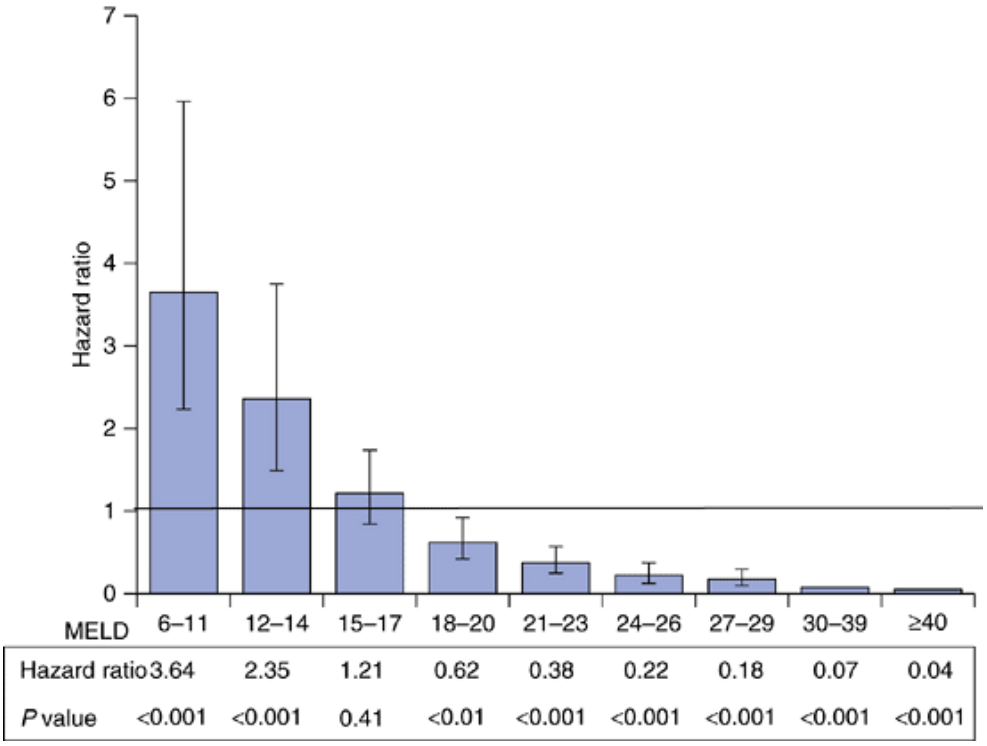


▪ **Figure 53.3** Comparison of mortality risk expressed as hazard ratio by Model for End-Stage Liver Disease (MELD) score for recipients of liver transplants compared to candidates on the liver transplantation waiting list. (From Merion RM, Schaubel DE, Dykstra DM, et al. The survival benefit of liver transplantation. *Am J Transpl* 2005;5:307–313.)

More recently, several reports have suggested that there are diagnostic groups of patients with chronic liver disease for whom MELD may not accurately reflect mortality risk. Patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfection (31), patients with intestinal failure (32), and perhaps patients with severe ascites (33) may have higher mortality risks than their MELD score defines. Future refinements in mortality risk models with concerted collection of natural history data for these patients will be required to improve the selection of patients with these conditions for transplantation. Overall, for individual patients with severe symptoms or poor HRQOL but low mortality risks, the decision of performing transplantation must be carefully weighed by the treating physicians to determine whether there is sufficient justification to accept the greater hazards of death for potential improvement in HRQOL.

Other Disease Progression Endpoints

For some patients with liver diseases treatable by transplantation, mortality or HRQOL risk may not be the correct metrics by which need can be judged. Examples of such conditions are hepatocellular cancer (HCC), metabolic liver

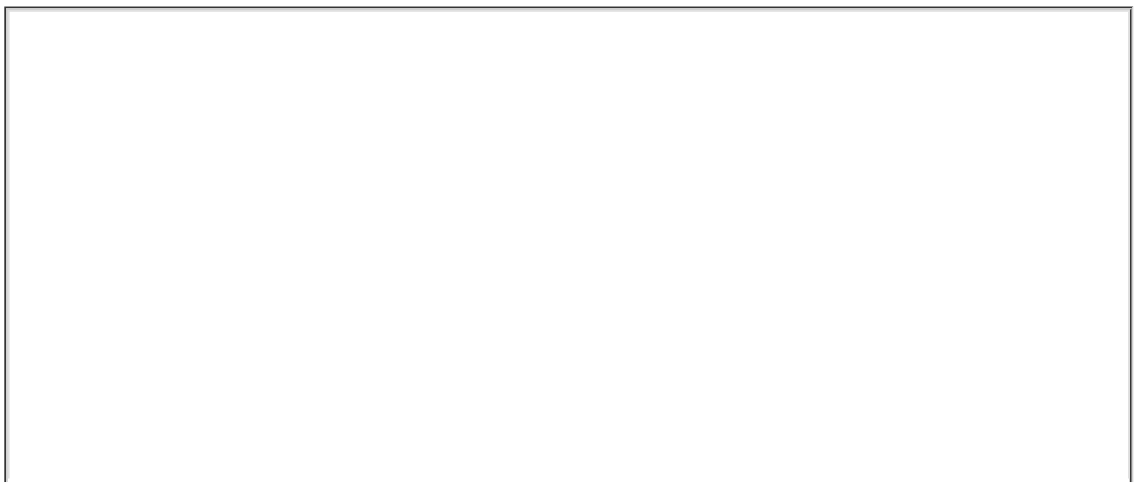
diseases, hepatopulmonary syndrome (HPS) and portopulmonary hypertension (PPH) syndrome. Patients with these disorders usually do not have significant mortality risks from their underlying liver disease but face risks of disease progression beyond a point at which liver transplantation can be offered with a reasonable chance of success. Unfortunately, risk models for disease progression for these

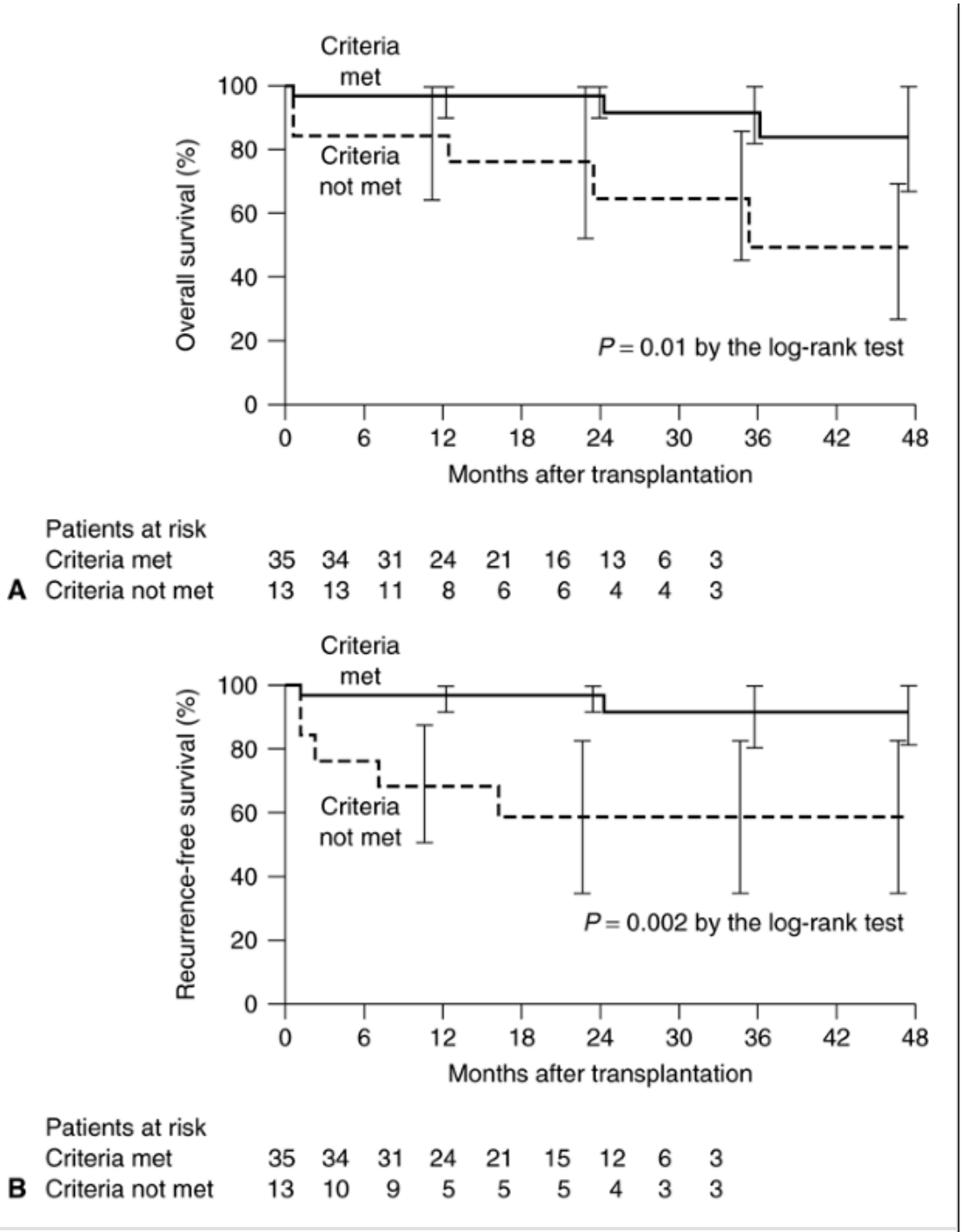
conditions have not been derived, making evidence-based selection problematic.

Hepatocellular cancer

Because HCC is increasingly recognized as a worldwide health problem (34), much more attention has been focused on its natural history and treatment. Liver transplantation was originally attempted to treat patients with extensive unresectable HCC (35). More recently excellent short- and longer-term results have been achieved with liver transplantation for patients with small HCC lesions arising in cirrhotic livers. Specifically, patients with cirrhosis and a single HCC lesion less than 5 cm in diameter or three or fewer lesions, the largest of which is less than 3 cm in diameter—the so-called Milan criteria—(36) (Fig. 53.4), have 4-year survival rates in excess of 80%, which are comparable to liver transplant recipients with nonmalignant primary liver diseases (37) and better than those undergoing surgical resection (38). These excellent results are dependent on the timing of transplantation in patients before the tumor extends to a larger size and/or disseminates. Therefore, selection of appropriate candidates with HCC for liver transplantation depends on the development of prognostic models of tumor progression. Investigators from Barcelona pointed out that the time spent waiting for a deceased donor liver was the most important determinant of HCC progression

beyond the Milan criteria (39), and it was subsequently found that application of adjuvant local treatment to HCC lesions for candidates waiting for transplantation for more than 1 year is a cost-effective strategy for maintaining these patients' candidacies within the Milan HCC criteria (40). More recent single-center studies have suggested that the probability of progression for HCC tumors that are less than 3 cm in size is approximately 10% within 1 year after listing, whereas larger tumors have an approximately 60% chance of progressing beyond the Milan criteria within a year of listing (41) (Fig. 53.5). Additional studies have been published indicating that lesions slightly beyond the Milan size criteria may have similar long-term results after transplantation (42,43,44), but these have not yet been confirmed in larger multicenter reports.



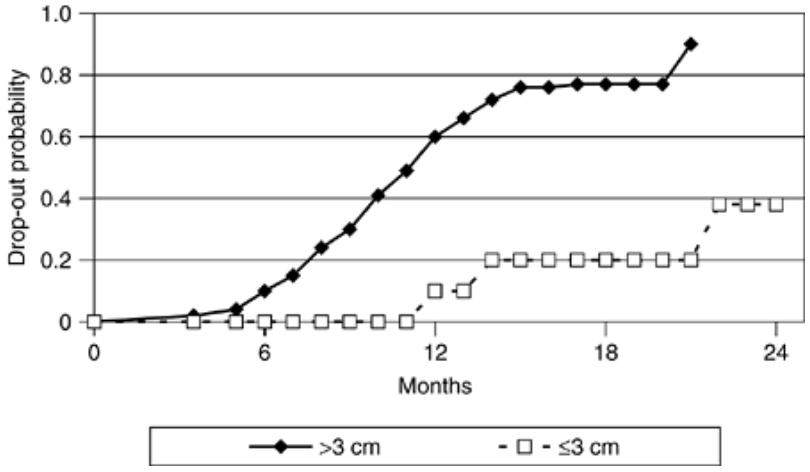


▪ **Figure 53.4** Correlation of post-transplantation pathologic confirmation of early stage hepatocellular carcinoma with overall survival **(A)** and recurrence-free survival **(B)** among 48 patients with cirrhosis. Data on the three patients who died within 1 month of transplantation were included in the calculation of recurrence-free survival. Before transplantation, all the patients were estimated to have either a single hepatocellular carcinoma 5 cm or less in diameter or no more than three tumors, each of which was 3 cm or less in diameter. After transplantation, the explanted livers were examined pathologically, and the patients whose tumors actually met the predefined criteria were compared with those whose tumors did not meet those criteria. Ninety-five percent confidence intervals (*bars*) are shown at 1-year intervals. (From Mazzafero V, Regalia E, Doci R, et al. Liver

transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996;334:693–699.)

For patients with HCC but minimal or no underlying liver disease, liver transplantation remains controversial. Some recent reports suggest that for patients with HCC and mild liver disease within CTP class A, long-term patient survival rates are improved with liver transplantation compared with liver resection (45). However, because of reasonable success for resection (46,47) and scarcity of organ donors, in addition to good success rates reported for salvage transplantation in patients with recurrent HCC after surgical resection (48), most authors advise surgical resection or ablation as a first-line treatment for HCC in patients with well-compensated liver disease (49,50).

Diagnostic accuracy for HCC, however, confounds the optimal timing of liver transplantation for these candidates. Many recent studies have shown that screening high-risk populations for HCC and subsequent confirmatory computed tomography (CT) or magnetic resonance imaging (MRI) can achieve sensitivity and specificity of 70% to 80% at best (51,52,53). The accuracy of imaging tests is further reduced for lesions less than 2 cm in size (52). Liver biopsy has been advocated to overcome some of these problems (54), but risks of bleeding, needle tract seeding by tumor, and sampling errors have limited its widespread use (55). Liver transplantation for patients with false-positive diagnoses of HCC who otherwise do not have severe underlying liver disease exposes these patients to increased surgical and immunosuppressive risks relative to medical management and diverts scarce organs away from those who could benefit more (See “Timing of Liver Transplantation in the Context of Liver Allocation”).



▪ **Figure 53.5** Probability of removal from the transplantation waiting list according to initial size of hepatocellular cancer tumor, comparing tumors presenting with more than 3 cm in size (*solid line*) with tumors 3 cm or less in size (*dashed line*). (From Yao FY, Bass NM, Nikolai B, et al. A follow-up analysis of the pattern and predictors of dropout from the waiting list for liver transplantation in patients with hepatocellular carcinoma: implications for the current organ allocation policy. *Liver Transpl* 2003;9:684–692.)

The available evidence suggests that patients with documented HCC within the Milan criteria who are otherwise acceptable liver transplantation candidates should be selected for the procedure. Diagnostic inaccuracy compels caregivers to confirm the diagnosis with complementary modalities (56). In the future, improved genomic or proteomic testing may be widely available and provide much more accurate diagnostic (57,58) and prognostic (44) information to optimize selection of patients for surgical resection or transplantation.

Pulmonary syndromes

Some patients with chronic liver disease develop pulmonary symptoms that impair their HRQOL and increase their risk for surgical intervention (59). These patients may or may not have severe intrinsic liver disease (60) and may not have advanced CTP or MELD scores (61). HPS defines a constellation of symptoms including dyspnea, hypoxia, reduced diffusing capacity of lungs for carbon monoxide (DLCO), and increased pulmonary arteriovenous shunting in the presence of chronic liver disease. Although there are no large-scale studies to adequately define mortality risk or even

precisely define the disease, most practitioners agree that patients with severe HPS have increased risk of death beyond that predicted by their MELD score and that they face increased operative morbidity, length of hospital stay, and transplantation procedure mortality (60,62). Results for patients selected for transplantation have been acceptable, with most patients experiencing improvement or resolution of their pulmonary condition (60,62). For these reasons, patients with signs and symptoms of HPS who do not have severe pulmonary hypertension should be considered for transplantation before their pulmonary function deteriorates to a point where anesthetic and surgical risks

become prohibitive.

Individuals with cirrhosis can also present with pulmonary hypertension or so-called PPH. In some cases these patients are asymptomatic, and their increased pulmonary resistance is discovered only at the time of invasive monitoring for the transplantation procedure. Patients with PPH have an increased mortality risk, above that expected from their underlying liver disease, and increased perioperative cardiovascular mortality has been reported for patients with elevated pulmonary artery pressures who undergo liver transplantation (62,63). Nonetheless, many of these cases can be managed effectively and outcomes for selected candidates, although sparsely reported, have been reasonable (62,64). These patients have to be carefully selected with multidisciplinary evaluation by transplantation hepatology, pulmonology, anesthesiology, and surgical specialists. Patients with elevated pulmonary artery pressures who respond to intravenous prostaglandin treatment have better results with liver transplantation than those who do not, making a trial of prostaglandin treatment a reasonable diagnostic and therapeutic choice before selection for transplantation (65,66). At this time, the available literature suggests that patients with moderate PPH or those who respond to prostaglandin treatment should be selected for liver transplantation because their outcomes are acceptable and transplantation may improve their PPH (67) afterward.

Metabolic liver diseases

Individuals with metabolic liver disease may also have legitimate indications for liver transplantation but may not have significant synthetic liver failure. Diseases such as Wilson disease, porphyria-induced liver disease, hemochromatosis, cystic fibrosis, and α_1-antitrypsin disease generally cause cirrhosis, portal hypertension, and hepatic synthetic failure. Consequently, mortality risk and existing measures of HRQOL should function well for these patients because they can be selected for transplantation using a mortality risk–based need for transplantation, defined by their intrinsic liver disease. However, patients with conditions such as familial amyloid polyneuropathy (FAP), hereditary oxalosis (HO), and inborn errors of hepatic metabolism, such as urea cycle defects, Crigler-Najjar syndrome, tyrosinemia, and other rare enzymatic diseases, may not develop hepatic fibrosis, portal hypertensive symptoms, or deterioration in hepatic synthetic function, as is normally captured by measurements of bilirubin levels, coagulation factors, or albumin synthesis, or assessment of portal hypertensive signs and symptoms. Nonetheless, particularly for metabolic defects that are intrinsic to the liver, liver transplantation has offered excellent short- and long-term results for properly selected individuals. The prime factor in selecting these patients for transplantation is whether the effects of the disease will be reversed by liver transplantation regardless of the anatomic location of the metabolic defect.

In adults, the best example of such a condition is FAP, in which the enzymatic defect usually occurs in an otherwise normal liver but causes severe systemic problems because of deposition of a mutant transthyretin (TTR) protein. Deposition of these fibrils in neurologic, cardiac, gastrointestinal, and urinary tissues results in progressive loss of function and an untreated median survival of 9 to 13 years (68). Liver transplantation restores normal TTR synthesis, with disappearance of the mutant protein from the blood of affected recipients and slowing or partial resolution of symptoms in successful cases (69,70). Therefore, the extent of secondary manifestations of FAP at the time of presentation, especially myocardial dysfunction and progressive neuropathy, limits successful

recovery after liver transplantation. Optimal selection of liver transplantation candidates with FAP requires early identification and assessment of the severity of end-organ involvement so that the candidate has sufficient cardiopulmonary reserve to survive the surgery and before these complications become so debilitating that they are irreversible. In some cases, the end-organ disease does not improve after restoring normal TTR synthesis with transplantation (68).

In children, patients with primary hyperoxaluria type 1 (PH-1), develop severe renal calculi and resultant renal failure, but the metabolic defect resides in the alanine glyoxylate aminotransferase gene that is exclusively expressed in hepatic peroxisomes. Therefore, liver transplantation cures the enzyme defect because the deficient genes are replaced by the transplantation of the normal liver, although established renal damage does not resolve after liver transplantation. Therefore, most patients with PH-1 are selected for liver transplantation after conservative measures have failed and renal disease has progressed. Consequently, many patients with PH-1 are treated with combined liver–kidney transplants (71). Preemptive liver transplantation, performed before renal complications have

progressed, has been advocated for patients with PH-1 (72), but the morbidity and mortality risks incurred by liver transplantation and immunosuppression must be considered carefully for these minimally symptomatic patients with relatively preserved renal function who may enjoy a good HRQOL and survival with conservative management for many years (73).

Other examples of diseases intrinsic to the liver are the urea cycle disorders (74,75), other hyperammonemic syndromes (76,77), maple syrup urine disease (78), and hereditary tyrosinemia type 1 (79), in which defects cause extremely elevated ammonia levels, neurologic disease, and coma, all of which can be reversed with liver transplantation if recognized early. In some cases, the neurologic consequences of these diseases progress to a point where they cannot be reversed by liver transplantation (75), making early recognition of these diseases the most critical aspect in selection of patients with these conditions for liver transplantation.

Pediatric Considerations

As alluded to in the preceding text, selecting children for liver transplantation is complicated by the fact that growth retardation, delayed development, and neurologic impairment are all deleterious sequelae of many of the liver diseases and metabolic disorders presenting in childhood. There is evidence that liver transplantation can help reverse some of the growth retardation (80,81) and developmental delays (82), but not completely (83,84,85). For these reasons, pediatricians have emphasized the need to select children for liver transplantation at a stage early enough in their disease that will provide a reasonable chance for some catch-up growth and development (83,86). There are several studies suggesting that children who receive transplantation have good HRQOL afterward (85,87), but these desirable outcomes must be weighed against the mortality risks of the surgery and immunosuppression because children who do not survive the transplantation procedure cannot possibly achieve catch-up growth, accelerate their development, or improve their HRQOL.

Mortality risk factors for children with liver disease differ from mortality risk variables in adults. The Pediatric End-Stage Liver Disease (PELD) score (Table 53.1) utilizes bilirubin level, international normalized ratio (INR), albumin level,

age, and growth failure factors to predict 3-month mortality with reasonable accuracy in patients younger than 19 years (17). Other prognostic scores such as the Wilson Disease Index employ white blood cell count and serum AST levels in addition to albumin, bilirubin, and INR values (88). Because children often present with diseases for which liver transplantation can be beneficial but without intrinsic liver synthetic failure, such as metabolic diseases discussed in the preceding text, selection of these candidates requires early diagnosis and identification of extrahepatic manifestations before they become irreversible.

Acute Liver Failure

Acute liver failure, also known as *fulminant hepatic failure* (*FHF*), remains a vexing and lethal clinical problem for pediatric and adult liver specialists. The diverse etiologies and difficulty in predicting which patients will recover spontaneously and those who will die without timely liver transplantation contribute to the complexity of selecting these patients for transplantation. Liver transplantation remains the best option for long-term recovery, but predicting who will recover without liver transplantation and who will not remains the critical issue for clinicians caring for these patients. Several prognostic scores have been developed to assist in decision making in this regard. The King's College group (89) and a French group (90) each published models for the prediction of death from acute liver failure (Table 53.2). Both these models have excellent positive predictive value for determining which patients will die from acute liver failure, but in subsequent studies both models have been shown to have relatively low negative predictive value (91,92,93). Although it may be desirable to err on the side of transplantation when the consequences of the failure to perform transplantation in a patient destined to die of liver failure are so extreme, the use of these models can result in inappropriate utilization of donor organs for patients who would otherwise recover without engraftment. More recently, in a study in which patients with acetaminophen toxicity were excluded, the MELD score was shown to have a superior positive and negative predictive value and a higher concordance for predicting which patients would die and which would recover with acute liver failure (94). These results have been further supported by a recent study of the US liver transplantation database in which the MELD score for patients with non–acetaminophen-induced acute liver failure was highly predictive of

mortality risk (95). Prognostic indicators for early mortality in patients with acetaminophen toxicity such as acetaminophen plasma half-life levels (96), coagulation factor levels (97), Gc protein levels (98), Acute Physiology and Chronic Health Evaluation (APACHE) II score (99) or phosphate levels (100), or addition of serum lactate (101) to the King's College models have been reported, but a recent meta-analysis found that these prognostic models have limited sensitivity and specificity, making them of questionable usefulness in the selection of patients with acetaminophen-induced liver failure for transplantation (102) (Table 53.3).

Table 53.2. King's College and Clichy Criteria for Liver Transplantation

King's College criteria INR >6.5 or any three of the following:

Age <10 or >40 y
Etiology: Drug toxicity or viral hepatitis
Duration of jaundice before onset of encephalopathy >7 d
INR >3.5
Bilirubin >17.5 mg/dL

Clichy criteria

Portosystemic encephalopathy
Factor V: <20% age <30 y
<30% age >30 y

INR, international normalized ratio.

Table 53.3. Results of the Meta-Analysis of the Prognostic Models for Acute Liver Failure

Criteria	No. of studies included	Positive likelihood ratio	Negative likelihood ratio
King's College[a,b]	6	12.33	0.29
pH <7.30[a]	4	7.44	0.48
PT >100 s + creatinine >300 µmol/L + encephalopathy grade ≥3	3	7.30	0.48
PT >100 s	3	2.05	0.40
Creatinine >300 µmol/L	2	1.91	0.50
APACHE II score >15	1	16.4	0.19
Increase in PT d 4	1	4.1	0.66
Factor V <10%	1	1.73	0.33
Gc-globulin <100 mg/L	1	Infinity	0.70

[a]Likelihood ratios on pooled measures of sensitivity and specificity are based on the final summary receiver operating characteristic model.
[b]King's criteria are pH <7.30 or a combination of PT >100 s + creatinine >300 μmol/L + encephalopathy grade ≥3.
PT, prothrombin time; APACHE, Acute Physiology and Chronic Health Evaluation.
From: Bailey B, Amre DK, Gaudreault P. Fulminant hepatic failure secondary to acetaminophen poisoning: a systematic review and meta-analysis of prognostic criteria determining the need for liver transplantation. *Crit Care Med* 2003;31(1):299–305.

Accurate prognostic information is essential for selection of patients with acute liver failure for transplantation. The King's, Clichy, and perhaps the MELD score may be helpful tools for patients with non–acetaminophen induced fulminant failure, but their low negative predictive value should be kept in mind. Selection of patients with acetaminophen-induced liver failure remains more problematic, and careful clinical observation for encephalopathy and trends in hepatic synthetic function remain indispensable in selection of these patients and all those with acute liver failure.

Timing of Liver Transplantation

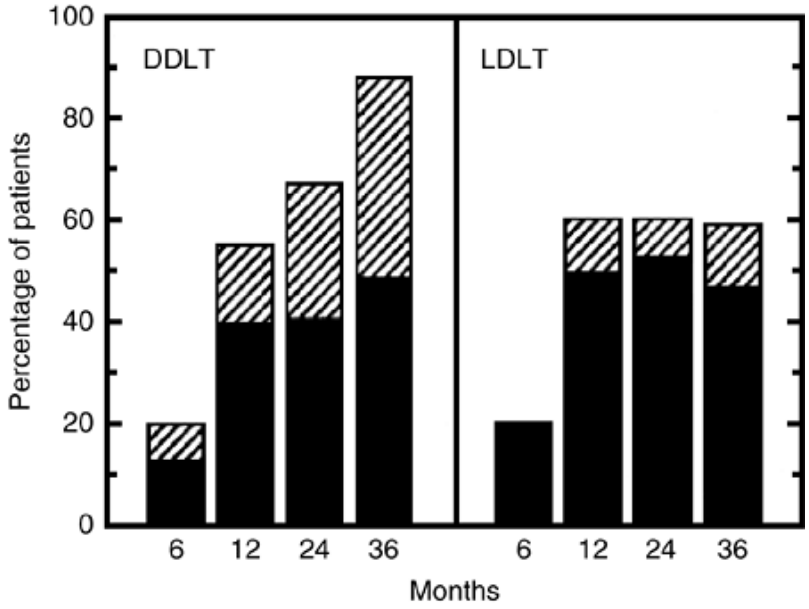
Living Donor Liver Transplantation

The advent of living donor liver transplantation in the late 1980s for children (103) and early 1990s for adults (104) introduced the possibility of electively timing liver transplantation. With this innovation, it became possible for patients with liver disease treatable with liver transplantation who are fortunate enough to have a suitable and willing donor to receive their transplant at a point where the risks of the surgery are outweighed by the risks of not performing the transplantation. This was the main justification for application of living donor liver transplantation to children (105) because pediatric deceased donor organs are even more scarce than adult organs and, as discussed previously, the timing of transplantation for children may not always be best estimated by mortality risk derived from the intrinsic liver disease. Patients selected for living donor liver transplantation have included children with most indications for liver transplantation, including acute liver failure, and adults with HCC (106,107) and other conditions that do not consistently receive enough priority on the waiting list. In general, for nonacute cases, living donor liver transplantation has been reserved for patients with less severe liver disease, and estimations of mortality risk can be helpful in selecting candidates who will derive benefit from the transplantation but who do not carry extreme post-transplantation mortality risks (108). A report from Japan used the MELD score to identify candidates who could receive the smaller left-lobe graft and still achieve an acceptable outcome (109). Controversy remains about the utility of living donor liver transplantation for patients with hepatitis C, with some reports suggesting more frequent and rapid recurrence (110,111) and others finding no difference (112,113) in recurrence rates compared with deceased donor grafts (Fig. 53.6).

Although there are limited reports of living donor liver transplantation being

performed for acute liver failure in adults (114), most clinicians would reserve the living donor procedure for patients with moderately severe chronic liver disease, in which there is sufficient time for a complete donor evaluation and informed consent without the added coercion of an acute, potentially immediate fatal liver disease in the recipient candidate. Conversely, living donor liver transplantation for pediatric candidates, in which an adult donor donates a

smaller portion of his or her liver and thereby faces a lower operative risk, seems applicable for almost all indications for liver transplantation in children.



▪ **Figure 53.6** Percentage of patients with fibrosis in their liver allograft at various intervals after deceased donor liver transplantation (DDLT) or living donor liver transplantation (LDLT). *Solid bars* represent portal fibrosis. *Hatched bars* represent bridging fibrosis. None of the patients in either group developed cirrhosis during the follow-up period. No significant difference in the percentage of patients who developed fibrosis existed between the two groups. (From Shiffman ML, Stravitz RT, Contos MJ, et al. Histologic recurrence of chronic hepatitis C virus in patients after living donor and deceased donor liver transplantation. *Liver Transpl* 2004;10:1248–1255.)

Paramount to selection of recipient candidates for living donor liver transplantation is a thorough assessment of the donor's risks for donation, estimation of the potential of success for the recipient, and fully informed consent from both donor and recipient.

Timing of Liver Transplantation in the Context of Organ Allocation

In an ideal world, all patients would receive the most effective treatment at the time when they are most likely to gain the most benefit and suffer the least harm from that intervention. However, because liver transplantation is severely limited by the availability of the therapeutic substrate, there must be some method for selection, from all those who could potentially benefit, of the few individuals who

will receive the treatment. Therefore, the timing of liver transplantation for individual patients will be determined in large part by this allocation/selection process regardless of the presence of a beneficial potential that a transplantation may pose for any of the waiting patients. Living donor transplantation introduces some flexibility for the timing of these cases, but because only approximately 5% of liver transplantation procedures in 2004 were from living donors (115), the timing of most liver transplantation procedures depends on the allocation system. Timing for these patients is not so much determined by what is most appropriate for that individual to maximize success and minimize complications and/or failures but by what is the most equitable for all users of the organ donor pool. Equitability is difficult to define, but prioritization of the measures of equitable outcomes is possible. Because deceased individuals cannot have a quality of life or growth or development, or otherwise improvement in their burden of disease, it is difficult to accept any form of liver allocation in which these nonmortality endpoints supersede the risk of death. Consequently, prioritizing an individual with a very poor quality of life or delayed growth and development before someone with a higher mortality risk is difficult to justify. However, risk of disease progression, as is conceptualized in liver allocation for patients with HCC in the United States, can also serve as an equitable prioritization tool. Therefore, timing of deceased donor liver transplantation will depend mostly on which individual from among all the waiting candidates has the highest mortality risk (or risk of disease progression) if he or she continues to wait although there may be many other individuals on the list for whom transplantation at that point of time would also be beneficial.

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Chapter 54

Immunosuppression: The Global Picture

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Key Concepts

- Advances in immunosuppression in recent years have led to impressive patient and graft survival rates and reduced rejection rates in liver transplantation.
- Initially immunosuppressive regimens included combination therapy with corticosteroids and azathioprine. This was followed by the introduction of antilymphocyte globulin, cyclosporine, FK506, mycophenolate mofetil, and rapamycin. A number of cytokine antibodies antagonistic to specific targets in the antigen recognition pathway, such as OKT3 and antithymocyte globulin, are also in use to help prevent rejection.
- Therapy with calcineurin inhibitors (cyclosporine or FK506) in combination with corticosteroids, azathioprine or mycophenolate mofetil is a popular regimen. Although used as induction therapy and in the treatment of rejection, use of corticosteroids has been declining especially in long-term transplant recipients. A major side effect of immunosuppression is the development of calcineurin inhibitor nephrotoxicity.
- Regular biochemical monitoring with drug levels and renal function is essential to ensure adequate immunosuppression and to prevent the development of immunosuppression-related side effects.
- The development of immunosuppressive agents that are less nephrotoxic may help decrease the incidence of renal failure in the long-term.

Outcomes after liver transplantation have shown consistent improvement in the recent years. Three and 5-year patient survival rates following deceased donor liver transplantation are 78% and 72% respectively, with graft survival slightly lower at 72% at 3 years and 64% at 5 years post-transplantation (1). These impressive patient and graft survival rates have been due to advances in surgical techniques and immunosuppression over the years. The use of combination therapy with corticosteroids and azathioprine by Thomas Starzl in the early 1960s brought about initial success in renal transplantation (2). This initial regimen was followed by the use of antilymphocyte globulin (ALG) in the late 1960s and early 1970s. The discovery and subsequent introduction of cyclosporine in 1979 represented a major breakthrough in the field of immunosuppression and resulted in continued improvements in patient and graft survival in organ transplantation. Immunosuppression in organ transplantation has been further refined by the

development and introduction of other agents such as FK 506 (tacrolimus), mycophenolate mofetil (MMF), rapamycin and a number of cytokine antibodies targeted against specific mediators in the allograft rejection pathway.

The goal of clinical immunosuppression is to provide graft acceptance with minimal side effects. However, the use of most immunosuppressive agents is associated with significant adverse effects requiring close monitoring and modification of therapy. An alternative strategy to immunosuppression in organ transplantation is to

try and induce tolerance in the recipient. Tolerance is the state of host nonresponsiveness to the transplanted organ and is induced by previous exposure to the antigen. The induction of tolerance may allow reduction in the level of immunosuppression and in some instances, may lead to its complete withdrawal. Although a few centers are involved in tolerance inducing protocols, currently there are no completely successful protocols and the induction of tolerance remains the holy grail of transplantation. In the absence of true tolerogenic protocols, immunosuppression remains the key to successful organ transplantation.

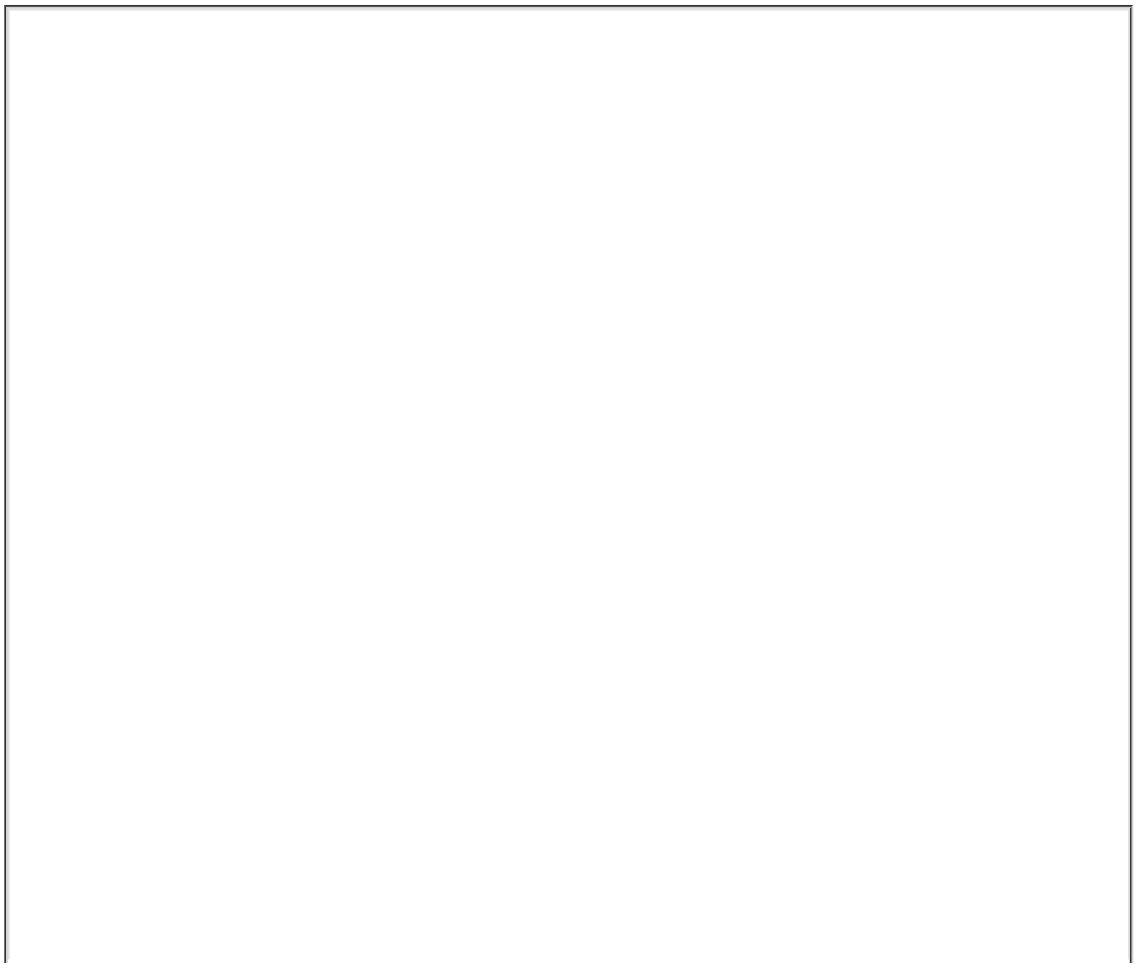
Overview of the Immune Response and General Features of Immunosuppression

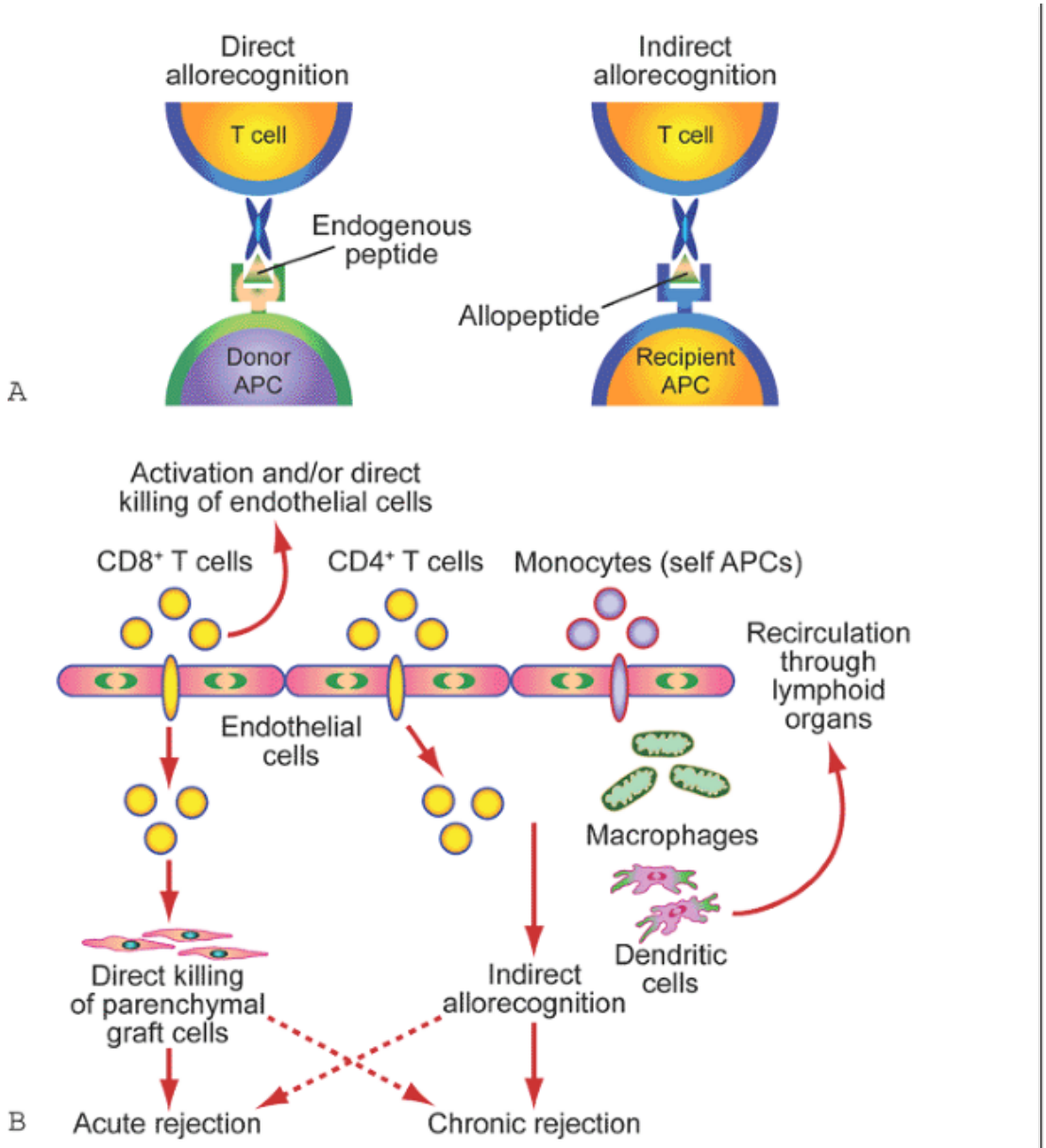
T cells respond to peptide antigens bound to the groove of class I (CD8 T cells) or class II (CD4 T cells) major histocompatibility complex (MHC). Recognition of foreign alloantigens is carried out by the recipient T lymphocytes through one of two pathways (Fig. 54.1). Donor hepatic antigen-presenting cells (APCs) express MHC molecules recognized by recipient T lymphocytes in the direct pathway. In the indirect pathway, recipient T lymphocytes react to donor alloantigen derived peptides expressed on recipient APCs. In liver transplantation, there is evidence that both these mechanisms exist (3). It is possible that the direct pathway is active during the early post-transplantation period, playing a major role in the development of acute cellular rejection. Here the APCs expressing donor antigens from the graft migrate and enter into secondary lymphoid tissue where they encounter allospecific T cells (4). These T cells, primed through the direct pathway, interact with the allograft and promote rejection. In the later stages, the indirect pathway may be important in sustaining a persistent response to the graft. This probably occurs as a result of donor antigen

derived from damaged donor liver being picked up and presented by self APCs resulting in the activation of T cells. Phagocytosis of apoptotic donor cells may be an important source of antigen for indirect presentation. However, the relative interplay and importance of the direct and indirect pathways in allograft rejection remain to be further clarified (5). Once the T-cell receptor, which is the antigen recognition unit, interacts with the MHC/peptide complexes on the surface of the presenting cell, accessory molecules including CD3 and CD4 or CD8 are brought into play. The T-cell receptor/CD3 complex interacting with the MHC molecule of the APC results in the activation of the T cell (signal 1). However, it is not known that more than one signal is needed to activate the immune system (6). Activation of the immune system by an antigen requires not only the antigen (signal 1), but also a signal from a secondary molecule (signal 2). It is also postulated that if a lymphocyte only received the signal from the antigen, it would not only fail to respond, but would also fall into a state of inactivity termed *anergy* (5). This forms the basis of the two-signal model by which full T-cell

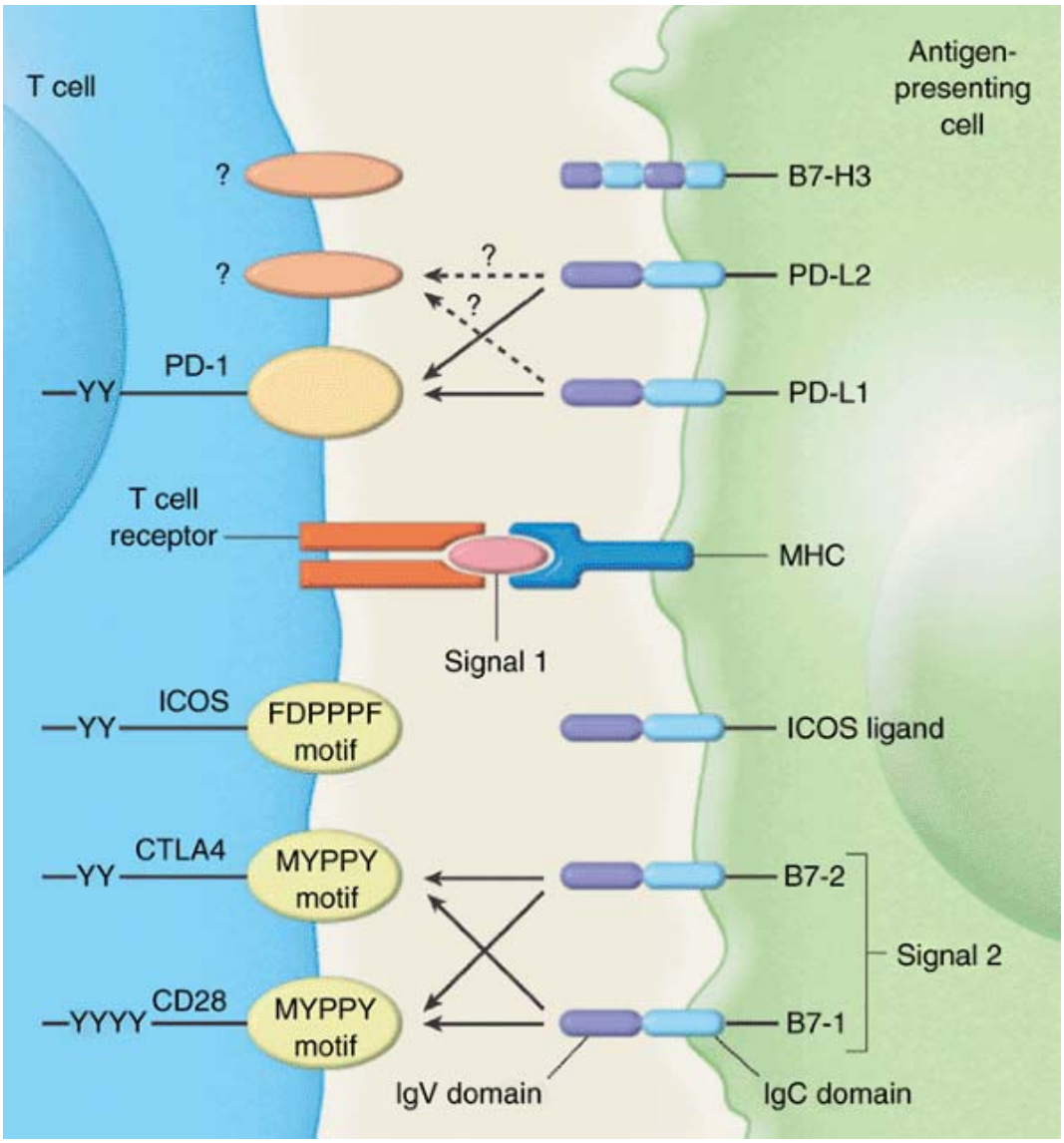
activation is dependent upon a complex array of regulatory molecules called *costimulatory* molecules (Fig. 54.2). Signal 2, a calcium-independent process, results from binding of the costimulatory molecules found on T cells with their ligands found on the APCs. Expression of costimulatory ligands on the APC is induced by factors released during tissue injury (so-called danger signals). Signal 1 and signal 2 activate an array of intracellular events mediated by calcineurin, protein C kinase, zeta associated protein-70, activation of nuclear factor of the activated T cells (NFAT), NF-κB, and activated protein (AP)-1 respectively. These factors migrate to the nucleus and bind to various gene promoters associated with T-cell activation and proliferation initiating the G0 to G1 transition in the cell cycle.

Further progression of T-cell activation results from autocrine and paracrine cytokine mediated signaling via specific cytokine receptors that include the interleukin (IL)-2 receptor family (often referred to as signal 3). An important cytokine is IL-2 that binds to the IL-2 receptor gamma chain and activates Janus kinase (JAK) 1 and 3. This triggers additional intracellular signaling pathways including signal transducers and activators of transcription (STAT) 5, Ras-Raf-MAP kinase, and mammalian target of rapamycin (mTOR)/P13-k/p7056 kinase (7). The cytokines generated by activated T cells facilitate the generation of effector cells such as antibody secreting B cells, cytotoxic T cells, and activated macrophages. Subsequently, these cells upregulate cell adhesion molecules such as CD2, LFA1, and VLA4 resulting in alteration of the migration pattern of cells. T cells also mediate cell damage by secretion of numerous factors such as tumor necrosis factor (TNF)-α, TNF-β (lymphotoxin), and fas ligand expression as well as cytotoxins such as perforin and granzymes F.





▪ **Figure 54.1** Allorecognition pathways and graft rejection. **A:** Pathways of allorecognition. In the "direct" pathway, T cells recognize intact major histocompatibility molecules on donor antigen-presenting cells (*left*). In the "indirect" pathway, T cells recognize processed alloantigen in the form of peptides presented by recipient antigen-presenting cells (*right*). **B:** Interactions among endothelial cells, T cells and recipient antigen-presenting cells in allograft rejections. The recipient monocytes are recruited by endothelial cells to the graft tissue. They are also transformed to become highly efficient antigen-presenting dendritic cells that may need to recirculate to peripheral lymphoid organs for maturation. The dendritic cells and intragraft macrophages present donor peptides via the indirect pathway to recruited $CD4^+$ T cells. $CD8^+$ T cells, on the other hand, are activated by donor endothelial cells or traverse the endothelium and kill parenchymal graft cells. APC, antigen-presenting cell. (Reprinted with permission from Briscoe DM, Sayegh MH. A rendezvous before rejection: where do T cells meet transplant antigens? *Nat Med* 2002;8:220–222.)



▪ **Figure 54.2** The two-signal hypothesis. In signal 1, the major Histocompatibility complex on the antigen-presenting cell interacts with the T cell receptor. B7-1 (CD80) and B7-2 (CD86) on the antigen-presenting cell interact with the respective ligands, CD28 and CTLA4 (CD152). CD28 and CTLA4 share common motifs (MYPPY) that are essential for binding B7-1 and B7-2. Regulatory molecules, such as programmed death 1 (PD-1) and inducible costimulator, which have different motifs, enter the regulatory cycle by affecting experienced T cells. CD28 and CTLA4 have similar structures, but opposite functions. CD28 activates T cells, whereas, CTLA4 inhibits them. PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; MHC, major histocompatibility complex; ICOS, inducible costimulator; IgV, immunoglobulin V; IgC, immunoglobulin C. (Reprinted with permission from Ingelfinger JR, Schwartz RS. Immunosuppression—the promise of specificity. *N Engl J Med* 2005;353:836–839.)

Overview of Immunosuppressive Agents

Broadly, immunosuppressive agents consist of two types: Pharmacologic and biologic. Biologic agents comprise the antilymphocyte antibodies (both the monoclonal and the polyclonal) and anticytokine receptor antibodies. Pharmacologic agents consist of corticosteroids, cytokines, suppressive agents, and cell cycle inhibitors (Table 54.1). Immunosuppressive agents act by inhibiting the pathways of foreign antigen recognition, T-cell activation, and costimulation. There are also other agents that inhibit a variety of other processes in the immune recognition pathway or lymphocyte trafficking. In addition, there are a few agents wherein the exact mechanism of action is still unknown.

Table 54.1. List of Clinically Available Immunosuppressive Agents and Target Pathways

Immunosuppressive agent	Target pathway(s)
PHARMACOLOGIC	
Corticosteroids	Selective lysis of immature cortical thymocytes Blockade of cytokine gene transcription in APC
Cyclosporine (Sandimmune, Neoral, Gengraf)	Signal 1 transduction through TCR
Tacrolimus (Prograf)	Signal 1 transduction through TCR
Rapamycin/Sirolimus and Everolimus/SDZ RAD (Rapamune and Certican)	Signal 3 transduction through IL-2 receptor
Azathioprine (Imuran)	Inhibition of purine metabolism and DNA synthesis
Mycophenolic acid (CellCept, Myfortic)	Inhibition of purine metabolism and DNA synthesis
BIOLOGIC	
Anti-CD3 pan-T cell (Orthoclone OKT3)	Causes depletion and receptor modulation in T cells Interferes with signal 1
Antithymocyte globulin (ATGAM,	Causes depletion and receptor

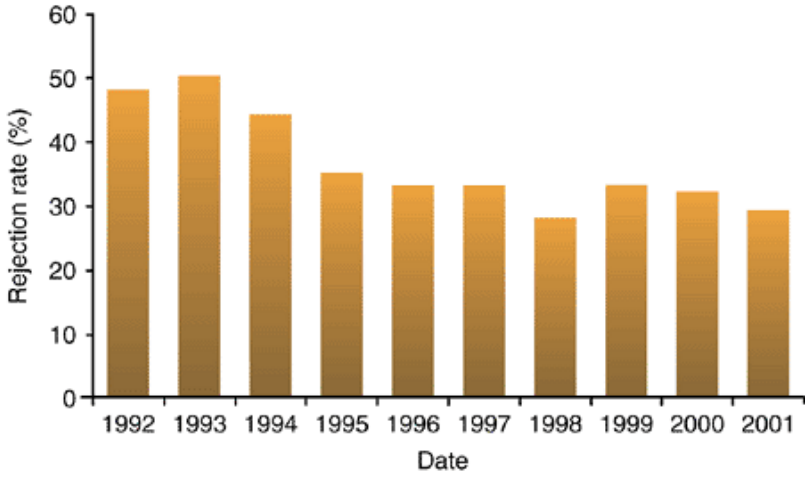
Thymoglobulin)	modulation in T cells Interferes with signals 1, 2, and 3 Inhibits lymphocyte trafficking
Anti-II–2 α-chain receptor (Zenapax, Simulect)	Inhibits T-cell proliferation to IL-2 (signal 3)
Anti-CD52 (Campath 1-H)	Causes depletion of thymocytes, T cells, B cells (not plasma cells), monocytes

APC, antigen-presenting cells; TCR, T cell receptor; IL, interleukin; DNA, deoxyribonucleic acid.

Corticosteroids were one of the earliest immunosuppressants used in the field of organ transplantation. Corticosteroids, in combination with the antimetabolite azathioprine, the earliest widely used immunosuppressive drug in transplantation, made renal transplantation a realistic option in the 1960s. Starzl et al. (2) and Murray et al. (8) showed independently that combining corticosteroids with azathioprine enabled prolonged survival of human renal allografts. Corticosteroids continue to play a major role in immunosuppression in liver transplantation. A recent report by the Scientific Registry of Transplant Recipients (SRTR) from an analysis from the United Network of Organ Sharing (UNOS) database revealed that corticosteroids were used in more than 90% of liver transplant recipients at the time of discharge (1). The use of azathioprine, however, has declined and only 4% of patients discharged from the hospital following liver transplantation were on this drug (1). Following the success of combination therapy with azathioprine and prednisone, the field of immunosuppression advanced in the late 1960s and early 1970s with the introduction of ALG and monoclonal anti–T-cell antibodies such as OKT3. However, immunosuppression finally came of age in the late 1970s with the description by Borel JF et al. of the striking effects of cyclosporine on the immune system, and the demonstration by Calne et al.

of the impressive advantage of using cyclosporine A in patients undergoing renal transplantation (9,10). Subsequently in 1989, FK506 (tacrolimus) was used in transplantation for multiple indications including treatment of persistent rejection with continued decrease in the incidence of rejection (Fig. 54.3) (11). Currently the field of immunosuppression is expanding with a number of agents with differing modes of action being studied for use in liver transplantation. Rapamycin which inhibits signal transduction, a different mechanism of action from tacrolimus which basically inhibits lymphokine synthesis, is currently being used in select patients in liver transplantation. MMF, a drug that blocks purine synthesis and salvage pathways is also being increasingly used in liver transplantation with approximately 50% of patients being on it at the time of discharge (1). Antibodies directed against T lymphocytes such as antithymocyte globulin or against specific cytokines such as the IL-2 receptor antibodies are also being increasingly used. A variety of investigational agents such as FK778,

WH1 P154 and FTY720 are also being tested (Table 54.2).



▪ **Figure 54.3** Trends in evidence of rejection at one year in liver transplant recipients, 1992–2001. (Reprinted with permission from Kaufman DB, Shapiro R, Lucy MR, et al. Immunosuppression: Practice and trends. *Am J Transplant* 2004;4(Suppl 9):38–53.)

Table 54.2. Investigational Immunosuppressive Agents in Clinical Testing and Target Pathways

Immunosuppressive agent	Target pathways
FK778	Interferes with pyrimidine metabolism and DNA synthesis
WHI P 154	Signal 3 transduction through JAK3/STAT5
LEA29Y	Signal 2—also known as CTLA4-Ig and inhibits B7/CD28 interaction
FTY720	Inhibits naïve T cell homing to the high venule endothelial cell on secondary lymphoid tissue

DNA, deoxyribonucleic acid; JAK, janus kinase; STAT, signal transducers and activators of transcription.

Corticosteroids

Corticosteroids were one of the earliest agents used for immunosuppression, first developed in the late 1940s and introduced into immunosuppressive regimens in the late 1950s (12). They have been applied in a variety of ways in liver transplantation. These include: (i) As induction immunosuppression by way of bolus corticosteroid therapy at the time of organ implantation, (ii) as maintenance therapy to prevent rejection, and (iii) in the treatment of established acute cellular rejection. Although their chronic, long-term use is somewhat disputed in recent years, they continue to be widely used both in induction therapy and during short-term maintenance therapy in liver transplantation. Corticosteroids have multiple effects and their exact mechanism of action is unknown. The most common agents used in liver transplantation include prednisone, prednisolone, and methylprednisolone. These steroid agents possess a predominantly anti-inflammatory immunosuppressive potency with relatively low mineralocorticoid potency. Prednisone is rapidly absorbed from the gastrointestinal tract; however, it needs to be metabolized to prednisolone for biologic activity. Following absorption, corticosteroids are primarily metabolized into inactive compounds by the liver and excreted in the urine. They exert their effects through their actions on a wide variety of white cells (including lymphocytes, granulocytes, macrophages, and monocytes) and endothelial cells. Glucocorticoids circulate either in the free form or in association with cortisol-binding globulin in the blood. The free form of the steroid readily diffuses through the plasma membrane of lymphocytes and binds with high affinity to intracellular glucocorticoid receptors. This ligand receptor complex, following activation, modulates transcription both positively and negatively, in the nucleus of specific genes

and coding factors critical in the generation and maintenance of the immune and the inflammatory responses. In addition to modulating transcription, glucocorticoids can also influence later cellular events including RNA translation, protein synthesis, and secretion (13). Leukocytes, by their mechanism of localization and activation, generate numerous products that play a key role in cellular rejection, and corticosteroids therefore, modulate this function. On lymphocytes the major mechanism of action of corticosteroids seems to be a negative regulation of cytokine gene expression. Regulation of cytokine gene production occurs through the inhibitory action of glucocorticoid on gene transcription through inhibition of AP-1 and nuclear factor NF-κB. Glucocorticoids also affect other cells such as macrophages, neutrophils, eosinophils, basophils, mast cells, and endothelial cells. Glucocorticoids antagonize macrophage differentiation and inhibit many of their functions that promote inflammation. Glucocorticoids inhibit neutrophil adhesion to endothelial cells, thereby decreasing their extravasation to the site of inflammation. This process may partly explain the neutrophilia that is seen with glucocorticoid therapy. However, neutrophil functions are only minimally influenced by glucocorticoids. In addition to their action on neutrophils and macrophages, glucocorticoids also decrease circulating eosinophil and basophil counts, inhibit IgE-dependent release of histamine and leukotriene from basophils and inhibit degranulation of mast cells. Additionally, glucocorticoids downregulate endothelial cell function including expression of class II MHC antigen, expression of adhesion molecule (ELAM-1) and the intracellular cell adhesion molecule (ICAM)-1), formation of IL-1 and arachidonic acid metabolites, thereby inhibiting the expression of cyclo-oxygenase type II.

Corticosteroids are administered at various stages of liver transplantion. High-

dose methylprednisolone is usually given intravenously around the time the liver is implanted, and continued for several days postoperatively. Induction doses range from 300 to 1,000 mg intravenously. Initially thought to be essential, trials have now demonstrated that corticosteroid-free regimens do not necessarily lead to increased rejection (14,15,16,17). These trials have however, used other induction agents such as antithymocyte globulin or IL-2 receptor antibodies. For maintenance therapy, steroids are rapidly tapered from the time of surgery to a daily maintenance dose of 5 to 10 mg per day. Subsequently, many centers now taper and stop prednisone after 3 to 6 months. Prednisone cessation does not seem to adversely affect the graft in most instances and may be beneficial in ameliorating some of the side effects of long-term prednisone use such as osteoporosis, diabetes, weight gain, hypertension, cataracts, hyperuricemia, and cosmetic problems. However, in patients who have undergone transplantation for autoimmune liver disease and in patients with conditions such as ulcerative colitis continuing prednisone therapy, albeit at a low-dose, may be prudent. Corticosteroids have also been used in the treatment of acute cellular rejection. In this context, intravenous methylprednisolone is given at a dose of 1,000 mg on alternate days for a total of 3 doses. The high-dose intravenous regimen for the treatment of rejection is usually stopped after this intensive protocol (18).

Table 54.3. Side Effects of Corticosteroids

Impaired wound healing
Hypertension
Weight gain
Hyperglycemia
Hyperlipidemia
Osteoporosis
Fluid retention
Hirsutism
Acne
Myopathy
Cataracts
Infection

Corticosteroid therapy is not without its side effects. Short-term corticosteroid use such as IV boluses to treat acute cellular rejection may cause transient hyperglycemia. Infections can also be unmasked or exacerbated by short-term high-dose corticosteroids. In patients with hepatitis C, the use of bolus high-dose intravenous corticosteroid therapy to treat acute cellular rejection has also been associated with a more severe and earlier recurrence of hepatitis C (19). Long-term corticosteroid therapy is associated with a number of side effects some of which may be reversed by steroid withdrawal (Table 54.3). Cosmetic side effects such as acne and hirsutism may adversely affect compliance especially in the younger population undergoing transplantation.

Azathioprine

Azathioprine was one of the earliest agents to be used in combination with corticosteroids in the long-term management of the transplant recipient, and has brought about a major improvement in graft survival. Azathioprine is absorbed

readily from the gastrointestinal tract, and peak plasma concentrations are achieved 1 to 3 hours after oral administration (20,21). Azathioprine is rapidly cleared from the circulation but approximately 30% is bound to serum proteins. Azathioprine is a derivative of mercaptopurine, which is rapidly metabolized to 6-mercaptopurine and subsequently to other active and inactive metabolites. The metabolites

are excreted through the urine with little intact drug excreted. The half-life of azathioprine is approximately 3 hours with normal kidney function and up to 50 hours in patients with anuria. The active metabolite acts as a purine analog, which is incorporated into cellular DNA and inhibits purine nucleotide synthesis and metabolism. The resultant alteration in RNA synthesis and function prevents mitosis and proliferation. Azathioprine acts early during the proliferative phase of cell cycle thereby inhibiting primary cell-mediated and humoral responses (22).

Azathioprine was first used in combination with corticosteroids for renal transplantation, and was found to be highly efficacious. It is currently used in combination with a calcineurin inhibitor and corticosteroids. Daily doses between 1 and 2 mg/kg are generally used in combination therapy. More lately, azathioprine has been replaced by MMF in a large number of transplant recipients as reported by the SRTR from their analysis of the UNOS database. Use of azathioprine at the time of discharge from the hospital was noted in only 4% of patients compared with 48% of patients on MMF.

Although used for a number of years, azathioprine has well-documented side effects. These include myelosuppression, reversible hepatotoxicity, alopecia, and gastrointestinal side effects such as nausea and dyspepsia, and acute pancreatitis. Other short-term side effects include the development of fever, skin rash, arthralgias, myalgias, and an acute hypersensitivity reaction. During long-term therapy an increased incidence of malignancies of the skin such as squamous cell carcinoma, and non-Hodgkin's lymphoma have been reported. Xanthine oxidase, a major enzyme in the catabolism of azathioprine metabolites is blocked by allopurinol and hence, when used concurrently with allopurinol the dose of azathioprine must be decreased to 25% to 33% of the original dose. Other drugs that interact with azathioprine and worsen its bone marrow suppression include methotrexate and angiotensin converting enzyme inhibitors.

Cyclosporine

Cyclosporine is a highly aliphatic cyclic undecapeptide. It was originally isolated from the soil fungus *cylindrocarpon lucidum* (9). Since its discovery, cyclosporine has remained the cornerstone of immunosuppression, not only for liver transplantation, but also for kidney, heart, and other organ transplantations. Cyclosporine came into widespread clinical use in the early 1980s in renal transplantation and was responsible for significantly improving early graft survival with some centers reporting almost doubling of their graft survival rate (10,23). In liver transplantation, Starzl et al. reported the first use of cyclosporine and prednisone in 1981 with 83% of their patients alive after 8 to 14½ months (23).

Cyclosporine enters cells and lymphocytes through diffusion and at high concentrations by active transport through the low-density lipoprotein (LDL) cholesterol receptor. In the cell, cyclosporine binds to a number of carrier proteins including cyclophilin, which are important for protein folding. The cyclosporine/cyclophilin complex binds to calcium activated calcineurin, a serene

threonine phosphatase important in the lymphocyte activation cascade, preventing the dephosphorylation of the transcription factor, NFAT. This prevents NFAT from engaging to specific DNA- binding sites in the promotor region of several T-cell growth factors and cytokines such as IL-2, interferon-γ, TNF-α, and costimulatory molecules such as CD40. This lack of engagement downregulates the expression of these various cytokines essential for the rejection process (24,25). Therefore, cyclosporine enables the activated cells to potentially return to their quiescent state inhibiting early antigen recognition, reducing clonal expansion, and inhibiting the synthesis of multiple cytokines necessary for rejection. Cyclosporine is not cytotoxic and does not inhibit the myeloid or the erythroid cell lines.

An earlier formulation of cyclosporine (Sandimmune) was notable for variable absorption and bioavailability. However, with the recent introduction of a microemulsion nonaqueous form (Neoral), absorption and bioavailability have become more predictable (26). Cyclosporine is absorbed primarily in the proximal jejunum, and achieves peak blood levels in 2 to 4 hours (26). It is widely distributed with highest concentrations found in adipose, kidney, adrenal, pancreatic, and liver tissues. Cyclosporine is metabolized primarily through the liver, occurring through the P450 system and excreted predominantly through the bile (22). Therefore, with liver failure, the propensity to develop toxic cyclosporine concentrations should be recognized. Cyclosporine has an average half-life of approximately 27 hours (range 10 to 40 hours). Various drugs that stimulate or inhibit the hepatic P450 enzyme system may increase or decrease dose levels and can result in underimmunosuppression or toxicity. These agents include the calcium channel blockers, antifungal agents, macrolide antibiotics, prokinetic agents such as cisapride and metoclopramide, and a variety of miscellaneous agents such as amiodarone, cimetidine, omeprazole, and protease inhibitors. Dosing of cyclosporine is generally regulated by examining a 12-hour trough level, as this is convenient to patients. However, it has been shown that trough concentrations do not accurately reflect the area under the curve for cyclosporine exposure in individual patients. More recently, the peak cyclosporine level at 2 hours after

oral administration is thought to be a better measure of the area under the curve, and may be more useful in controlling toxicity and enhancing efficacy. Target trough and peak levels vary with respect to time after liver transplantation. Within the immediate post-transplant period, 12-hour trough levels between 250 and 300 ng/mL are desirable. With time, however, 12-hour trough levels between 100 and 125 ng/mL may be sufficient to prevent rejection and minimize toxicities. Cyclosporine is commonly used in conjunction with prednisone and either azathioprine or MMF soon after transplantation. With time, the other agents may be withdrawn leaving cyclosporine as the sole immunosuppressant.

The main side effects of cyclosporine include nephrotoxicity and hypertension (27,28). With long-term follow-up of liver transplant patients, it is becoming apparent that 15% to 20% of the patients on calcineurin inhibitors will end up with chronic renal insufficiency requiring dialysis and/or renal transplantation (29,30). The nephrotoxicity of cyclosporine is thought to result from its vasoconstrictor effects on renal blood vessels. Although early toxicity resulting in renal dysfunction may be reversible, the late stages of cyclosporine nephrotoxicity resulting in advanced tubular interstitial fibrosis and scarring may be irreversible. Other significant side effects from cyclosporine include neurologic

sequelae, which occur in 10% to 20% of the patients (20). These include paresthesias, tremors, hallucinations, confusions, and migraine-like headaches. Other rare side effects may include seizures, especially in patients receiving intravenous cyclosporine. Cyclosporine may also cause hirsutism and gingival hyperplasia that causes significant cosmetic problems in the younger patient undergoing transplant (28). Other less commonly reported side effects include nausea, vomiting, thrombocytopenia, allergic reactions, tinnitus, myalgias, and arthralgias.

Tacrolimus

Tacrolimus (FK506) is a metabolite of a fungus *streptomyces tsukubaensis* and was isolated in 1986 (31). Its mechanism of action is identical to that of cyclosporine. When it enters the cell, it binds with an immunophilin referred to as FK-binding protein 12. This complex then inhibits calcineurin, preventing the dephosphorylation of the transcription factor NFAT and inhibiting the transcription of cytokines necessary for rejection (22). Tacrolimus is considered to be 10 to 100 times more powerful than cyclosporine. Its first successful use was reported by Starzl et al. in 1989 as rescue therapy for liver grafts failing conventional therapy (11). Subsequently, tacrolimus was evaluated for routine use in liver transplantation. A multicenter trial, that compared the efficacy and safety of tacrolimus with cyclosporine showed that tacrolimus was associated with significantly fewer episodes of corticosteroid resistant or refractory rejection, although graft survival and patient survival were not significantly different (32). Similar results were also seen in the European Multicenter Study; additional findings included a lower incidence of chronic rejection and infection. However, the use of tacrolimus in the US Multicenter Study was associated with a higher incidence of adverse events requiring withdrawal from the study, primarily nephrotoxicity and neurotoxicity. Tacrolimus has been proved to be safe and efficacious in long-term immunosuppression after liver transplantation with fairly low incidences of rejection and malignancies (33,34). Tacrolimus is absorbed in the duodenum and jejunum and, unlike cyclosporine, does not require the presence of bile (22). The recommended initial dose is 0.1 mg/kg/ per day every 12 hours. Initial levels after liver transplantation are maintained between 10 and 15 ng/mL. With time, 12-hour trough levels may be lowered to between 5 and 10 ng/mL to reduce side effects. Although initially used in combination with other immunosuppressive agents, most commonly corticosteroids and azathioprine or MMF, tacrolimus monotherapy in the later stages of liver transplantation is more widely practiced.

The side effects of tacrolimus are similar to those of cyclosporine (35). The most significant effects include nephrotoxicity and neurotoxicity. The neurotoxic side effects of tacrolimus include headaches, insomnia, tremors, dysarthria, seizures, and coma (36). It may be necessary to switch over from tacrolimus to cyclosporine therapy to obviate some of these side effects. The incidence of hypertension may be lower with tacrolimus. Tacrolimus also seems to be a more diabetogenic medication when compared with cyclosporine with up to 10% of the patients developing de novo diabetes mellitus (37). The other side effects include hyperlipidemia, nausea, diarrhea, abdominal pain, and pruritus.

Mycophenolate Mofetil

MMF is a prodrug that is rapidly hydrolyzed to its active metabolite, mycophenolic acid. It is a selective and noncompetitive inhibitor of inosine monophosphate

dehydrogenase, which is an important enzyme in the de novo pathway of guanine nucleotide synthesis (22,38). This results in the inhibition of DNA synthesis in T and B lymphocytes thereby inhibiting cell proliferation and function. Other cell types can use salvage pathways and are not affected; therefore, the effects of MMF are largely seen on the immune cells with few effects on the nonimmune system. Although long known to be an

immunosuppressive, it is only recently that its use has increased in liver transplantation.

MMF is metabolized to mycophenolic acid glucuronide which is excreted in bile and has an enterohepatic circulation. It is excreted in the urine in humans and to a lesser extent in the bile. There may be larger variations in pharmacokinetics in liver transplant recipients related to fluctuations in serum albumin, unlike that seen in renal transplant recipients (39).

Clinically, MMF is used mainly as a replacement to azathioprine in liver transplantation. It has similar side effects as azathioprine, but it is less myelotoxic and has fewer hepatotoxic side effects. It is usually used in combination with a calcineurin inhibitor and steroids. The use of MMF in conjunction with corticosteroids and tacrolimus has been shown to be superior in preventing rejection and improving renal function than the combination of tacrolimus and corticosteroids alone. Patients discharged home on immunosuppression that included MMF appear to have better long-term patient and graft survival (40). It is possible that the use of MMF may enable a lower dose of a calcineurin inhibitor to be used or withdrawn, in an attempt to preserve renal function (41,42,43). Therefore, MMF may be particularly useful in patients who may be at a higher risk of rejection such as younger patients undergoing liver tranplantation for fulminant liver failure and patients with autoimmune liver disease. MMF as monotherapy after calcineurin inhibitor withdrawal has been associated with a high incidence of acute cellular rejection and steroid-resistant acute cellular rejection (44,45). Therefore, the use of MMF alone may not be sufficient to prevent rejection after liver transplantation. The usual dose is 1,000 mg given orally twice daily. Dose-reductions are made depending on toxicity. An enteric-coated preparation (Myfortic) is available that allows delayed release of the active drug in the small intestine rather than the stomach. This may help alleviate some of the gastrointestinal side effects of MMF.

The significant side effects of MMF include gastrointestinal symptoms, mainly diarrhea, abdominal discomfort, anorexia, and bone marrow suppression including neutropenia. The incidence of side effects is high and dose dependent. Variations in pharmacokinetic from changes in serum albumin levels may increase the incidence of side effects if drug levels are high. Side effects may require dose-reduction and withdrawal in 25% to 57% of patients (46). Occasionally patients with gastrointestinal side effects may respond to increasing the frequency of dosage to three or four times daily. Gastrointestinal side effects may be reduced by acid reduction with a proton pump inhibitor. Bone marrow suppression usually responds to dose-reduction, but in some instances may require drug discontinuation.

Rapamycin

Rapamycin or sirolimus is a new macrolide antibiotic immunosuppressive agent. Rapamycin is structurally similar to tacrolimus. It is named after its isolation from the fungus, S*treptomyces hygroscopicus*, found in soil samples from Rapa

Nui or Easter Island brought back in 1968. However, its immunosuppressive properties were not reported until 1988. Like the calcineurin inhibitors, cyclosporine and tacrolimus, rapamycin acts by binding to an immunophilin FKB12. However, this does not affect calcineurin activity, but instead, the complex binds to and inhibits a protein called the *mTOR*. Inhibition of mTOR results in selective inhibition of synthesis of new ribosomal proteins which are essential for progression of the cells from the G1 to the S phase (47). In addition, rapamycin has also been associated with diminished fibroblastic activity by its actions on the 70 kDa S6 kinase in the fibroblast (48).

In liver transplantation, rapamycin is primarily used as an immunosuppressive agent for its renal sparing effect. In addition, it has some unique properties such as antiproliferative and antineoplastic effects, and hence may be useful in patients who undergo liver transplantation for hepatocellular carcinoma and cholangiocarcinoma. The usual regimen is to provide a loading dose, 5 mg PO, and continue with maintenance doses of 2 to 3 mg perday. It is usually used in the later post-transplant period and levels are maintained between 5 and 10 ng/mL.

Rapamycin has a number of side effects, which currently restricts its usage in liver transplantation. (49,50,51,52). In early trials in liver transplantation, rapamycin was combined with FK506 in the immediate post-transplant period in an attempt to reduce the incidence of renal dysfunction. However, when used with FK506, rapamycin was associated with a higher incidence of adverse events which included thrombotic events such as hepatic artery thrombosis, infection, and death (53,54,55). Hence, most centers use rapamycin in the later post-transplant period, usually after a period of 3 to 6 months. The adverse effects of rapamycin include thrombocytopenia, leukopenia, anemia requiring blood transfusions, arthralgias, hyperlipidemia, pneumonitis, and diarrhea (56). There have also been reports of wound complications in the immediate post-transplant period (57). This effect is presumably a result of its antilymphoproliferative effects on fibroblasts, thereby delaying wound healing. Oral ulcers were seen with the liquid preparation; however, this does not seem to be a problem with the pill preparation. In addition to the those mentioned in the preceding text, there have been reports of an increased risk of nephrotoxicity when combining rapamycin with high doses of calcineurin inhibitors (58).

An analog of rapamycin, RAD (Everolimus) is currently undergoing clinical trials in liver transplantation. It differs from rapamycin only in its bioavailability.

Antibody Therapies

Antibody therapies in liver transplantation may consist of either the polyclonal antibodies or monoclonal antibodies. Polyclonal antibodies include antithymocyte globulin and ALG. Polyclonal preparations may be obtained by immunizing either the horse or rabbit with different immunogens, whereas monoclonal antibodies are produced in response to a single antigen (59). The advent of hybridoma technology to fuse two cell lines, one capable of specific antibody production and the other capable of permanent cell growth, was a key breakthrough in monoclonal antibody production (60).

Polyclonal Antibodies

Polyclonal antibodies include antithymocyte globulin and ALG, which are prepared

by immunizing animals such as the horse or rabbit with immunogens from different sources. These immunogens may include lymphocytes, thymocytes, blast cells, or T cell lines. Antithymocyte globulin is produced by using human thymocytes as immunogens, whereas, ALG is produced by using human lymphocytes. Polyclonal agents have been in use since the early 1960s. The initial agents, termed *antilymphocyte serum* (ALS) consisted of unfractionated serum obtained from animals after immunization with human lymphocytes. Subsequently, this preparation was purified and the immunoglobulin was concentrated, as it was known that the immunosuppressive properties of the serum were contained in the immunoglobulin fraction. Various preparations of polyclonal antibodies are available throughout the world. In the United States, the commonly used preparations are antithymocyte globulins derived either from the horse (Atgam) or the rabbit (Thymoglobulin). The polyclonal antibodies exert their action through various mechanisms, which include complement mediated cell lysis, increased uptake of T cells by the reticuloendothelial system, and also by modulation of surface receptors of lymphocytes and blocking their function. The administration of polyclonal antibodies results in profound lymphopenia. Currently, they are not commonly used in liver transplantation. There have been reports of their successful use in corticosteroid-free protocols with comparable rates of rejection (14,15,16,17). In addition, they may have a role in the treatment of steroid-resistant acute cellular rejection.

In addition to profound leukopenia, administration of antibody therapy is associated with the cytokine release phenomena characterized by fevers, chills, hypotension, which may require admission to the intensive care unit (60,61,62,63). Other less common side effects include nausea, diarrhea, arthralgias, thrombocytopenia, dyspnea, and seizures. Concerns about an increase in the infectious complications and increase in the incidence of post-transplant lymphoproliferative disorders are also present with the use of these therapies.

Monoclonal Antibodies

Monoclonal antibodies are produced in relation to a specific antigen. These include the mouse monoclonal antibody muromonab (OKT3), which is specific to the CD3 receptor. Other antibodies include the IL-2 receptor antibodies that include daclizumab and basiliximab. In addition, other monoclonal antibodies such as Campath 18 (alemtuzumab) are also being investigated.

OKT3 has been the most extensively used monoclonal antibody in liver transplantation. It was originally used in 1987 for prophylaxis against acute cellular rejection (64,65). OKT3 is directed against the CD3-antigen complex found on T cells. Binding of OKT3 to this complex results in deactivation of the CD3-antigen complex and subsequent internalization of the T cell receptor, thereby preventing subsequent antigen rejection (66). Administration of OKT3 results in rapid depletion and extravasation of most T cells from the blood stream and peripheral lymphoid organs such as lymph nodes and spleen. This depletion is secondary to both cell death and margination of the T lymphocytes onto vascular endothelial walls and redistribution to nonlymphoid organs such as the lungs. OKT3 is currently used for the treatment of steroid-resistant cellular rejection in liver transplantation (61,62). It is associated with a high incidence of adverse events related to the cytokine release phenomena, and premedication with steroids and/or antihistamines is generally advised (63) (Table 54.4). This syndrome usually occurs within an hour of drug administration during the first 2

to 3 doses of OKT3. Additionally, during OKT3 administration, prophylaxis against infections such as cytomegalovirus (CMV), pneumocystis and fungi are also advised. The use of OKT3 may also be associated with early and severe recurrences of hepatitis C after liver transplantation (67), and hence, must be used with caution in this cohort of patients. The total lymphocyte counts and CD3 counts are usually monitored during therapy and dose adjustments may be needed to ensure adequate suppression of the CD3 count.

Table 54.4. Side Effects of OKT3

Fever
Hypotension
Chills
Headache
Rash
Nausea
Vomiting
Chest pain
Diarrhea
Pulmonary edema

Two IL-2 receptor antibodies, daclizumab and basiliximab, are currently being studied in liver transplantation. The exact mechanism of action is still incompletely understood, but likely results from their binding to the IL-2 receptor on the surface of activated, but not resting, T cells. They are fairly well tolerated and have a low incidence of side effects (58,68,69). They may be used for induction immunosuppression especially in patients with renal failure to provide a window of opportunity for the kidneys to recover before commencing calcineurin inhibitors. They have also been used for induction immunosuppression in corticosteroid-free protocols (70,71,72,73).

Alemtuzamab or Campath is a humanized recombinant anti-CD52 monoclonal antibody. Campath produced profound depletion of circulating lymphocytes for about several months with slow lymphocyte recovery (74,75). Experimentally it has been used in the treatment of multiple sclerosis with thrombocytopenia and Graves' disease observed as side effects. Campath has been used in liver transplantation in tolerance inducing protocols (76). Severe recurrence of hepatitis C has been noted. Currently, the use of Campath in liver transplantation is considered experimental and its use is confined to research protocols.

Newer Immunosuppressive Agents

The prevention of nephrotoxicity from calcineurin inhibitors remains a difficult challenge. The development of newer immunosuppressive agents that are less nephrotoxic may help prevent the development of renal dysfunction in the long-term. There are various agents currently being developed as immunosuppressive agents in liver transplantation. These include FK778, a synthetic analog of leflunomide which interferes with pyrimidine metabolism and DNA synthesis, FKY720 derived from *Isiria sinclaivii*, which alters cellular homing patterns of

lymphocytes, JAK inhibitors that inhibit activation of immune cells, and agents such as CTLA4 IG and betacelept that cause costimulation blockade (77,78,79,80,81,82,83). These agents are all considered investigational and are currently undergoing clinical trials.

Goals of Immunosuppression

The goals of immunosuppression are to control alloreactivity to eliminate acute rejection, but not adversely influence the development of recurrent hepatitis C, minimize side effects, preserve renal function, and reduce costs. In the absence of satisfactory markers of overall immunosuppression, tolerance and rejection/alloreactivity, and poor correlation between rejection and the degree of liver enzyme elevations or immunosuppression levels, immunosuppression in transplantation still remains an art. The key is to tailor immunosuppression to the individual patient. In patients with renal insufficiency at the time of liver transplantation, calcineurin inhibitors are withheld and an IL-2 receptor blocker may be used as an induction agent. Calcineurin inhibitors are introduced once renal function has recovered. In the event of non-recovery of renal function, the addition of rapamycin may be considered. Switching to rapamycin may also be considered in patients who develop renal dysfunction while on calcineurin inhibitors. An alternative to this regimen would be to consider adding MMF and lowering the dose of the calcineurin inhibitor. Patients with complications from long-term corticosteroid use may benefit from being weaned off from corticosteroids in a controlled fashion while carefully monitoring for rejection. Caution must be exercised before embarking on this protocol in patients who have undergone liver transplantation for autoimmune liver disease such as autoimmune hepatitis, primary sclerosing cholangitis or primary biliary cirrhosis, and in younger patients to prevent recurrent disease or rejection.

Conclusion

Calcineurin inhibitors in conjunction with steroids and MMF continue to play a major role in immunosuppression following liver transplantation. Efforts are currently underway to develop newer immunosuppressants with less nephrotoxic effects, which will help reduce the incidence of long-term renal complications in these patients. In future, the development of these newer agents with less nephrotoxicity, and advances in inducing tolerance in the recipient will enable prolonged survival in patients undergoing liver transplantation.

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In this registry study, liver transplant recipients discharged home on immunosuppression that included MMF appeared to have improved long-term patient and graft survival.

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Chapter 55

The First 6 Months Following Liver Transplantation

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Key Concepts

- Clinical assessment immediately postoperatively in the liver transplant recipient focuses on hemodynamic stability and multiorgan function, including neurologic assessment and renal function, which reflect graft function. Early profound graft dysfunction suggests primary nonfunction or hepatic artery thrombosis (HAT), which necessitate prompt retransplantation
- Care in the early weeks of post-transplantation care involves monitoring graft function for acute cellular rejection (ACR), prophylaxis against opportunistic infections such as cytomegalovirus (CMV), and management of immunosuppression and its side effects.
- Immunosuppression initially involves of calcineurin inhibitors (CNIs) (tacrolimus or cyclosporine) and corticosteroids, transitioning to maintenance regimens that reduce or eliminate corticosteroids. Adjunct agents such as mycophenolate mofetil or sirolimus are added in some cases, particularly if there is renal insufficiency
- Significant side effects of immunosuppression including diabetes, hypertension, and hyperlipidemia need to be anticipated. Recommendations for routine health maintenance issues must be reinforced.
- ACR becomes less of a concern as patients progress further from the time of transplant but differentiation of rejection from recurrent disease can become a challenge during the initial 6 months. Chronic ductopenic rejection may evolve following ACR. Other important causes of late graft dysfunction include late recognition of HAT.

Intensive Care Unit Management

Following liver transplantation (LT), the focus of medical care immediately shifts from managing the manifestations of decompensated liver disease to monitoring liver graft function, anticipating complications such as rejection, maintaining effective immunosuppression while minimizing its side effects, and caring for a patient recovering from complex major abdominal surgery.

On return from the operating room to the intensive care unit (ICU), close clinical and hemodynamic monitoring is essential. Careful attention is paid to the patient's level of consciousness, urinary output, and hemodynamic status. Although less frequently used than previously in donor-recipient bile duct anastamosis, a T-tube allows easy assessment of bile output with pale scanty bile implying poor graft function.

Assessment of the graft function in the initial 24 to 48 hours after transplantation may be somewhat difficult, as elevated serum aminotransferases and other biochemical evidence of graft injury due to a variety of factors including graft ischemia and reperfusion injury are the norm. Serum aminotransferases may be markedly elevated initially but should rapidly decline if graft function is satisfactory along with normalization of prothrombin time in the first several days post-LT. Simple clinical observations including alert mental

status, adequate urine output, and hemodynamic stability are also reflective of good graft function. There are two major causes of profound graft dysfunction in the immediate postoperative period, which require prompt recognition: Primary graft nonfunction and hepatic artery thrombosis (HAT). Importantly, hyperacute rejection due to preformed antibodies is extremely rare post-LT. Primary graft nonfunction (PNF) occurs in 7% to 10% of allografts and is manifested by persistent coagulopathy, encephalopathy, poor bile production, and elevated serum aminotransferases in the immediate postoperative period. Risk factors for PNF include a reduced-size graft, donor graft steatosis greater than 20%, older donor age, retransplantation, renal insufficiency, and prolonged cold ischemia times (1). Recognition of PNF is an indication for prompt retransplantation.

HAT complicates 7% of adult LTs (range 4% to 25%), and 10% to 40% of pediatric cases (2). Risk factors include technical and anatomic difficulties, including small size of donor hepatic artery and complex hepatic artery anastomosis. Clinical signs of HAT resemble PNF, also reflecting profound graft dysfunction, if HAT occurs in the first several days following LT. Color Doppler ultrasonography is performed to confirm blood flow in the hepatic artery. The sensitivity of ultrasonography is reported to be approximately 90% in detection of acute HAT but angiography may be needed for definitive diagnosis. Late complications of unrecognized HAT, reflect ischemia of the biliary tree and include biliary strictures and bilomas. In common with PNF, HAT in the early postoperative period is an indication for prompt retransplantation although occasionally surgical thrombectomy may be successful.

Infectious precautions, including oral antibiotic prophylaxis for gut decontamination and intravenous antibiotics, are taken before the transplantation and in the operating room. Intravenous antibiotic prophylaxis should be continued for at least 24 hours after transplantation, and should cover a broad spectrum of organisms, specifically gram-positive skin flora and enteric gram-negative organisms. Opportunistic infections are not a major concern in the first few weeks following LT if graft function is good. Infections are typical of any patient recovering from major abdominal surgery, notably wound and pulmonary infections. Along with assessment of allograft function, routine ICU care including ventilatory management with weaning and pulmonary toilet as indicated. Other important issues at the bedside include monitoring of output from the Jackson-Pratt abdominal drains placed at the time of surgery to exclude a bile leak or intra-abdominal bleeding before their eventual removal, incentive spirometry once extubated, gradual advancement of oral intake as tolerated, and pain management. Once stable, transfer to a ward where the staff is experienced in the care of LT recipients is important for continuity of patient care.

Therapeutic immunosuppression is divided into induction (initial immunosuppression) and maintenance immunosuppressive phases, with additional immunosuppression for treatment of acute cellular and chronic ductopenic rejection. A number of immunosuppressive agents are currently employed (3). Newer immunosuppressive agents have often undergone evaluation initially in renal transplant recipients, reflecting the greater need for effective antirejection regimens in other areas of solid organ transplantation. Although rejection cannot be discounted in LT, the more formidable threat to ultimate graft viability is recurrent disease. An uncomplicated episode of acute cellular rejection (ACR) conveys a survival advantage in many LT recipients reflecting a relatively well-preserved immune system in contrast to renal transplant recipients in whom it results in diminished graft survival (4). The primary goal of immunosuppression is to prevent graft rejection and loss; a secondary goal is to avoid the adverse consequences of antirejection therapy. Immunosuppression is initiated intraoperatively with a steroid bolus. Although regimens vary by center, steroids are continued at a high dose for the first several days following LT while the mainstay of modern immunosuppression, a calcineurin inhibitor CNI, is introduced within the first postoperative day. Increasingly mycophenolate mofetil is added to the regimen as an adjunct immunosuppressive agent (which can allow for calcineurin sparing) in the setting of preexisting renal insufficiency or acute oliguric renal failure. Rejection is generally not a concern for the first week to 10 days post-LT.

Floor Care

Clinical care following transfer of patient to the floor is aimed at continued close monitoring of graft function, along with titration of immunosuppression into therapeutic range, wound care, early ambulation or physical therapy as tolerated, and patient and caregiver education.

Graft function in the days subsequent to discharge from ICU should continue to improve with resolution of coagulopathy and biochemical dysfunction (Table 55.1). Some clues to a likelihood of continued early allograft dysfunction that may portend a poor worsened long-term patient and graft survival include recipient prothrombin time or bilirubin that stays persistently elevated, donor age greater than 50 years, cold ischemia time greater than 15 hours, and donor preprocurement acidosis (5). Rejection also becomes an increasing concern in this phase of post-LT period. Any significant elevation in liver enzymes should prompt a diagnostic

liver biopsy. Biopsy-proven acute rejection occurs in up to 75% of LT recipients, typically within the first year.(6). Balancing adequate immunosuppression with the side effects of the CNIs, especially renal toxicity, is important (Table 55.2). ACR occurs in up to 70% of liver transplant recipients in the first year, of these 68% had both biochemical and histologic abnormalities consistent with ACR, while 32% had only histologic findings on protocol biopsies, without biochemical abnormalities. The latter is of unclear significance (7). ACR becomes most frequent after the first week. Most patients are clinically asymptomatic despite early mild acute rejection; however, hepatomegaly and tenderness to palpation of the allograft can be seen in late or severe acute rejection. Elevations in total bilirubin, transaminase (aspartate transaminase [AST] and alanine transaminase [ALT]), γ-glutamyl transpeptidase (GGT), and/or alkaline phosphatase levels, after initial normalization post-LT, suggest, but are not specific for, ACR which requires confirmation by liver biopsy as histopathology remains the gold standard for its diagnosis. Rejection is suggested by the presence of portal inflammation, bile duct damage, and venous subendothelial infiltration by inflammatory cells (Table 55.3). In many cases, the presence of eosinophils can also be a helpful indication of acute rejection (8). However, cellular rejection can be difficult to distinguish from recurrent disease, especially recurrent hepatitis C infection, although the latter does not usually occur for at least several weeks post-LT. Importantly, treatment of apparent ACR with steroid boluses is a predictor of more severe recurrent hepatitis in recipients with hepatitis C virus (HCV) infection, so the distinction is not merely of academic interest (9). ACR is typically treated with increased immunosuppression, initially with a corticosteroid bolus and/or an increase in the dosage of maintenance immunosuppressant medication (such as tacrolimus) especially if blood trough levels are found to be subtherapeutic. Antilymphocyte therapy such as antithymocyte immunoglobulin or the monoclonal antibody OKT3 may be used in the setting of steroid refractory rejection.

Table 55.1. Graft Function and Dysfunction in the Liver Transplant Recipient

	First week	Second and third week	Third to twelfth week	Third to sixth month
Physiologic	Aminotransferase normalize INR diminishes to normal Discharge from intensive care unit	Continued improvement in hepatic and renal function Discharge from hospital	Normal liver chemistries	Normal liver function
Pathologic	Hyperacute rejection PNF HAT	Acute cellular rejection Impaired graft function	Acute cellular rejection Cytomegalovirus Recurrent HCV Delayed HAT	Recurrent HCV Biliary stricturing due to HAT Recurrent primary sclerosing

				cholangitis

INR, international normalized ratio; PNF, primary graft nonfunction; HAT, hepatic artery thrombosis; HCV, hepatitis C virus.

Table 55.2. Common Side Effects of Immunosuppression

Tacrolimus	Cyclosporine	Prednisone
Nephrotoxicity	Nephrotoxicity	Osteoporosis
Tremor	Tremor	Osteonecrosis
Hypertension	Hypertension	Diabetes
Headache	Headache	Hyperlipidemia
Gastrointestinal symptoms	Hirsutism	Hirsutism
Alopecia	Gingival hyperplasia	Hypertension
Diabetes		Cushingoid habitus

Cytomegalovirus (CMV) is a major cause of morbidity and mortality in the first 14 weeks after transplantation before effective routine prophylaxis against and was implicated in other adverse outcomes such as chronic rejection (CR) (10,11) Particularly at risk of primary CMV infection are seronegative recipients of allografts from seropositive donors. Other risk factors include retransplantation and the use of antilymphocyte antibodies. CMV infection may manifest as asymptomatic viremia or overt disease with multiorgan involvement of skin, gastrointestinal tract, and graft dysfunction. Intravenous ganciclovir (6 mg/kg/day) given for 100 days postoperatively reduced the risk of CMV infection to 5% to 10% (12). Recent studies comparing oral ganciclovir (after a 14 day intravenous induction) to intravenous ganciclovir until day 100 after transplant showed no statistical difference in 1 year CMV infection rates, obviating the need for long-term intravenous access (13). Clinical disease is generally treated with intravenous ganciclovir. Although resistant strains of CMV occur, clinical disease typically reflects inadequate prophylaxis rather than CMV resistance.

Pneumocystis carinii (*PCP*) infection occurs in 6% to 20% of patients undergoing solid organ transplantation in the absence of prophylaxis. Trimethoprim-sulfamethoxazole (TMP/SMX) is effective in preventing

PCP and is generally given for 6 to 12 months after transplantation at the time of highest immunosuppression and greatest risk. Intolerance to TMP/SMX may then require second line

agents including aerosolized pentamidine or atovaquone.

Table 55.3. Rejection Activity Index and Grading of Acute Liver Allograft Rejection

(A) Rejection Activity Index (RAI)
Criteria that can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document

Category	Criteria	Score
Portal inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils, and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile duct inflammation damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells; more than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity, and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not most of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3
Total RAI Score = _/9		

From (7a) *Hepatology*. Banff schema for grading liver allograft rejection: an international consensus document. 1997;25(3):658–663.

(B) Grading of acute liver allograft rejection
Global assessment of rejection grade made on a review of the biopsy and after the diagnosis of rejection has been established

Global assessment[a]	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see reference in subsequent text)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As in preceding text for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

[a]Verbal description of mild, moderate or severe acute rejection could also be labeled as Grade I, II and III, respectively.
From (7a) *Hepatology*. Banff schema for grading liver allograft rejection: an international consensus document. 1997;25(3):658–663

Neurologic dysfunction can present during this phase of hospitalization as acute delirium, seizures, hallucinations, and tremor. The differential diagnosis includes metabolic causes (uremia, electrolyte imbalance), infection, residual encephalopathy, and medication side effects. Steroids and CNIs have known neurologic side effects, and switching from tacrolimus to cyclosporine or adding an adjunct medication such as mycophenolate mofetil to reduce the CNI dose may be of benefit. Any localizing symptoms should be assessed with prompt imaging to rule out focal hemorrhage or infarct. Central pontine myelinosis (CPM) is a neurologic finding associated with variable symptoms that can include coma, dysarthria, dysphagia, ophthalmoplegia, quadriparesis, tremor, locked-in syndrome, and ataxia among others. Development of CPM can be associated with rapid sodium shifts in the perioperative period, which lead to intramyelinic edema and subsequently demyelination in the pons. CPM has been reported in 5% to 12% of orthotopic liver transplantation (OLT) autopsy series, however, is more rarely seen in clinical practice. Prevention includes minimizing serum sodium fluctuations and avoiding excessively high levels of CNIs.

Mild to moderate ascites after transplantation is common but usually resolves over days to weeks. Conservative management with continued moderate salt restriction and the judicious use of diuretics as tolerated by renal function usually leads to fairly rapid resolution of the ascites. If large volume ascites persists, investigation should be initiated toward hepatic vein

and atrial pressure measurements to detect pressure gradients. Rodes et al. found that factors contributing to large volume ascites post-OLT included inferior vena cava preservation with piggyback technique, suggesting difficulty in graft blood outflow (14).

Other medical issues during the course of hospitalization include the management of systemic hypertension and diabetes. Hypertension is the most common cardiovascular complication following liver transplantation occurring in up to 50% to 80% of recipients. The relative risk of ischemic cardiac events was 3.07 in allograft recipients compared to an age-matched population without transplantation (15). Mechanisms for hypertension include altered vascular

reactivity and vasoconstriction related to CNIs, impaired glomerular filtration rate (GFR) and sodium excretion, and the effects of steroids. Treatment of hypertension should counteract sodium and water retention due to CNIs. A thiazide diuretic may be effective if used judiciously to volume depletion. Vasoconstriction due to CNI use is also a factor in systemic hypertension, so vasodilatation with a calcium channel blocker is appropriate first-line therapy. Diltiazem and verapamil increase the CNI levels and have more limited use than nifedipine. Angiotensin-converting enzyme (ACE) inhibitors, due to aggravation of renal dysfunction and hyperkalemia, are generally not recommended in the early post-transplantation course but may be useful in transplant recipients with diabetes, in the long term. Poorly responsive hypertension may require addition of a β-blocker or a centrally active agent such as clonidine.

Impaired glucose tolerance and diabetes are common in patients with cirrhosis due to peripheral insulin resistance, which predisposes them to post-transplantation diabetes (PTDM). HCV infection also enhances the risk of PTDM. The diabetogenic effect of immunosuppressives, mainly tacrolimus and cyclosporine as well as that of corticosteroids, are important factors. Treatment of diabetes in OLT recipients is based on the same principles as in the nontransplant patient. Treatment with a regular insulin sliding scale while hospitalized to assess insulin needs, then discharge on oral medications and/or insulin is usual. Insulin requirements should lessen as reduction in corticosteroids and other immunosuppressive medications occurs. Diet, lifestyle modification, obesity reduction, and the continued use of insulin or oral hypoglycemic agents may be indicated; with glyburide being an initial choice if an oral agent is felt to be sufficient. Metformin is best avoided because of concern about lactic acidosis in the presence of renal dysfunction that is common in this population.

Patient and caregiver education is the final key to a successful hospitalization for OLT. A thorough discussion with a member of the transplantation team on medication indications, side effects, and interactions is important in the assurance of patient compliance. Patients should be made aware of “warning signs” for which an immediate call to the transplantation center is warranted. Emphasis is placed on good general medical care, use of sunscreen because of an increased risk of cutaneous malignancies, and age appropriate health screening.

Early Clinic Visits

Frequency of outpatient clinic visits vary, but usually are weekly in the first 1 to 2 months, biweekly thereafter, and monthly for up to 1 year. Goals of the clinic visit include assessment of the patient's overall health and well being, activities of daily living, medication compliance, and biochemical and immunosuppression monitoring. In the first weeks following transplantation, a continued low threshold for suspicion of rejection needs to be maintained, and liver biopsy has to be obtained for any significant elevation on serum aminotransferases. Elevations in bilirubin and/or alkaline phosphatase or features of obstruction on liver biopsy suggesting an obstructive clinical picture should prompt a cholangiogram. The cholangiogram may be performed through the T-tube, if present, or increasingly by magnetic resonance cholangiopancreatography (MRCP) if a Roux-en-Y anastomosis was performed. Endoscopic retrograde pancreatography (ERCP) may also be performed by experienced biliary endoscopists but risks of perforation or pancreatitis must be considered. Biliary imaging can distinguish between an anastomotic stricture versus the more worrisome intrahepatic biliary stricturing, which may reflect hepatic arterial occlusion leading to biliary ischemia or use of a suboptimal graft with, for instance, protracted cold ischemia.

Anastomotic strictures are usually amenable to biliary stenting whereas intrahepatic stricturing may require retransplantation because of its frequent association with HAT or other graft dysfunctions and lack of success with stenting. Nonanastomotic strictures in addition to ischemia can reflect other problems such as prolonged cold ischemia time, ABO incompatibility, and more recently the use of organs procured for transplantation from non–heart beating donors.

CMV infection should still be suspected if diarrhea or pneumonitis occur with fever and graft dysfunction, especially if prophylaxis has been discontinued. The diagnosis of CMV infection is confirmed by culture of tissue or blood with rapid tissue culture techniques using indirect immunofluorescence. Treatment is with high-dose ganciclovir.

Immunosuppression levels continue to be monitored with fasting levels drawn of specific CNIs, such as cyclosporine or tacrolimus. Serum drug levels may vary based on diet and concomitant medication usage

and need to be adjusted accordingly. Duration of steroid taper varies by transplantation center, but most recipients are weaned to low or no steroids by 6 months post-OLT.

Hyperlipidemia, with a mixed profile of elevated cholesterol and triglyceride levels, is noted in up to 30% of OLT recipients. Elevated lipid levels may be associated with allograft vasculopathy. The etiology of hyperlipidemia is multifactorial including obesity, preexisting disease, and medication induced. Cyclosporine causes inhibition of bile acid synthesis, and sirolimus and corticosteroids have been shown to cause dyslipidemia. Treatment of obesity and diabetes as well as dietary and lifestyle modifications are first-line measures. If serum lipid levels do not fall, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors can be safely prescribed. Myositis, rhabdomyolysis, and hepatotoxicity are uncommon side effects. The choice of agent is dependent on cost, degree, and type of hyperlipidemia. Nicotinic acid, bile acid binders, and fibric acid derivatives are generally avoided because of their potential hepatotoxicity or interference with other medications. Importantly, many OLT recipients succumb to nonhepatic causes as time from transplantation increases, reflecting factors such as an increased risk of cardiovascular disease, neoplasia, and other factors such as calcineurin-induced renal failure (16). Meticulous attention to systemic hypertension and other risk factors for adverse outcomes therefore assume major significance in the care of the OLT recipient.

Late Clinic Visits

After the first several weeks of close follow-up by the transplantation team, good communication with the primary care physician should lead to a smooth transition to long-term care.

CR, although uncommon, usually occurs after the first 6 months. Patients with CR can be relatively asymptomatic but typically present with laboratory parameters of cholestasis, such as a rise in alkaline phosphatase, GGT, and/or total bilirubin levels. On biopsy, loss of small bile ducts and obliterative angiopathy are classic histologic features. Several risk factors contribute to CR. Almost all affected patients have had at least one episode of acute rejection. Other contributing factors are inadequate immunosuppression in the early postoperative phase, CMV infection, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), or a history of autoimmune hepatitis (17). CR redevelops in as many as 90% of patients who undergo retransplantation for this reason (18). A number of reports have implicated interferon therapy for recurrent HCV infection in CR, as is discussed elsewhere in this book (see Chapter 58). Steroid boluses and antilymphocytic treatment, although efficacious in the treatment of acute rejection, have little effect in CR. Tacrolimus has proven to be the most effective treatment of CR, especially if the diagnosis of CR was made within 3 months of transplantation (19).

Other important causes of late graft dysfunction include recurrence of the original liver disease as exemplified by HCV (see Chapter 58); however, nonviral diseases including cholestatic and autoimmune hepatitis can also recur. In PBC, biopsy features typical of the disease in the native liver are observed whereas in PSC, biliary stricturing reminiscent of disease in the absence of OLT can also occur. Although recurrence of the cholestatic liver diseases occur less frequently than HCV, and previously hepatitis B virus (HBV), at least some grafts are lost and with long-term follow-up about one fourth of patients who underwent transplantation for cholestatic disease experience clinically overt recurrence. Possible factors implicated in disease recurrence include tacrolimus-based immunosuppression in PBC and absence of colectomy in PSC. No definitive strategy to prevent or ameliorate recurrent disease has emerged. In contrast, recurrent autoimmune hepatitis with reappearance of autoantibodies is generally steroid responsive. A de novo form of autoimmune hepatitis, especially in the pediatric population, may be more difficult to control. Recurrent and de novo nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are also recognized.

Osteopenia is common in patients with cirrhosis and typically is due to osteoporosis rather than osteomalacia, although the latter can occur in severely cholestatic patients. Factors implicated, apart from calcium and vitamin D deficiency include low muscle mass, immobility, long-term corticosteroid use, poor nutrition, and alcohol abuse. After transplantation, rapid bone loss occurs in the first 3 to 6 months, but ultimate recovery of bone mass can continue for up to 7 years after OLT. For patients awaiting OLT, serum calcium, phosphorus, thyroid

function studies, and parathyroid hormone should be evaluated along with bone densitometry studies. In addition to calcium and vitamin D, calcitonin or bisphosphonates may be considered if bone density indicates osteoporosis or if long-term corticosteroids are used. Once weekly oral dosing with alendronate or risedronate is well tolerated and convenient.

Skin cancer, lymphoma, and oropharyngeal cancer are more common in OLT recipients. Cigarette smoking is obviously prohibited. The patient should see a dermatologist on a regular basis. Patients with transplantation for PSC who have inflammatory bowel disease remain at high risk for colonic dysplasia and colon cancer, and require regular colonoscopy even in clinically quiescent disease.

Beyond 6 months, post-OLT recipients should be vaccinated for influenza, pneumococcus, and tetanus.

Live vaccines like measles, mumps, and rubella, and live oral polio vaccine should not be given.

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Chapter 56
The Long-Term Care of Transplanted Patient

Timothy M. Mccashland

Key Concepts

- Common medical problems in the long-term liver transplant recipient include obesity, osteoporosis, cardiovascular disease (CVD), and diabetes mellitus, all of which may result in increased morbidity and mortality.
- Technical related problems of biliary complications may be corrected by endoscopic therapy. However, living-related biliary complications are common both in the donor and the recipient and require a multidisciplinary team approach.
- Common causes of deaths in survivors for more than 1 year include development of de novo malignancy, cardiovascular causes, renal failure, and recurrent disease. Diligent surveillance for these diseases may lead to early detection and lower morbidity.
- Renal impairment is common in long-term survivors. Development of end-stage renal disease is reported in up to 18%, with markedly decreased survival in these patients.
- Recurrent hepatitis C leading to impaired allograft function is common. Treatment with combination therapy of pegylated interferon and ribavirin results in 20% to 35% clearance of the virus, but often requires growth factors and meticulous follow-up.

The traditional model of care in most transplantation centers is for the transplantation surgeon to manage the immediate postoperative care, with gradual incorporation of transplantation hepatologists and primary care physicians (1,2). With increasing success in liver transplantation, the need to manage these patients beyond the initial 6-month period often requires multiple providers and a larger commitment to prevent medical complications. Distinct differences exist among transplantation centers as to who becomes the primary physician in charge of long-term management (3). The transplantation hepatologist can provide expertise to encompass the overall care of common medical conditions, gastrointestinal- and transplantation-related problems. In a survey of transplantation centers, 40% of programs regarded the transplantation hepatologist as the primary care provider. An equal 40% had the primary care physician (family medicine/internist) as the principal provider of long-term management (3). Therefore, the management of long-term care seems to become the responsibility of internist/family physicians and transplantation hepatologists. Interestingly, transplantation surgeons and referring gastroenterologists were not expected to provide much input. Transplantation centers do not want primary care physicians managing immunosuppression, acute allograft rejection, recurrent disease, and biliary complications.

Several comprehensive reviews of medical complications and management of liver transplant recipients have been published previously (4,5,6,7,8). In 2006, the American Board of Internal Medicine will initiate a subspecialty board certification in transplantation hepatology of which 25% of the test will be related to the management of long-term care (9). This chapter will address management of long-term care of the liver transplant recipient divided into topics of quality of life (QOL), causes of death in long-term survivors, medical

problems (obesity, gout, vaccines, bone disease, cardiovascular problems, diabetes

mellitus), inflammatory bowel disease (IBD), pregnancy, and transplantation-related diseases (biliary complications, de novo neoplasia, renal dysfunction).

Quality of Life

The immediate goal of liver transplantation is to improve survival of the recipient. However, the secondary goal is to improve the QOL and return the patient to a productive life. Numerous studies have been published on this topic (10,11). Diverse results are likely due to different assessment tools and methods. Early studies with follow-up of less than 2 years noted liver recipients achieve very satisfactory levels of health-related quality of life (HRQOL). However, the HRQOL levels were below that of the general population (10). A meta-analysis confirmed these early findings (11). A prospective multicenter study assessing pretransplantation and post-transplantation HRQOL using the 36-item short-form health survey (SF-36) and EuroQol (EQ-5D) instruments with follow-up of 2 years showed a significant improvement in all dimensions of the SF-36 and role-emotion dimensions of the EQ-5D scores (12). Longer survival after transplantation, younger age, and patients from larger transplantation centers were independent predictive variables of higher QOL scores. Panter et al. studied patients up to 5 years after transplantation and found that the number of comorbid conditions and participation in physical activity were significant independent contributors to improved HRQOL (13). A recent French study compared QOL in survivors beyond 10 years after liver, kidney, and heart transplantation with the general population (14). The National Institute of Diabetes and Digestive and Kidney Disease transplantation QOL questionnaire was used to assess 315 patients (liver 126). Perceived physical health, personal function, and social and role functions were similar in the transplantation patients, but lower than the general public. Perceived psychological health was better than the general public in liver and heart transplant recipients but lower in kidney recipients. The QOL beyond 10 years in liver transplant recipients is similar to the general public according to this study. Moore et al. in a large, single-center study, found that functional performance and HRQOL were not affected by donor, recipient, or technical characteristics (15). Chronic immunosuppression, while it may contribute to some complications after transplantation, had little impact on HRQOL in one study (16). Jennings et al. noted no difference in QOL between Laennec's cirrhosis and non-Laennec's patients with follow-up to 5 years (17). However, another study at a mean follow-up of 4 years found impaired lower global QOL, physical functioning in hepatitis C patients with recurrent disease (18).

Psychological improvement after liver transplantation has also been shown to begin immediately after surgery. More recent long-term studies tend to show that depression-coping skills, anxiety, and social environments are more relevant in liver transplant recipient's QOL than other somatic factors (19,20,21). Not surprisingly, elevated levels of anxiety and neuroticism before transplantation were associated with worse psychological health after transplantation (22). A concerning and interesting investigation by Lewis et al. using multiple tests in cognitive function in 36 patients 10 years after transplantation showed that the patients scored significantly lower than healthy controls across a wide range of cognitive functions (23). Unfortunately, the reason for this lower function is unknown and needs further study. Blanch et al. using the psychosocial-adjustment-to-illness scale found that women had poorer adjustment to orthotopic liver transplantation (OLT) than men; the proportion of women with poor adjustment was higher (31.5% vs. 16.7%) (21). Women showed a greater dysfunction in health care orientation, sexual relations, and extended family relationships and psychological distress. Therefore, women may need more psychological intervention after transplantation than men. Therefore, psychological factors are important contributors to QOL issues in the long-term management of liver transplant recipients.

Causes of Death in Long-Term Survivors

According to the United Network of Organ Sharing (UNOS) database, the overall 1-, 3- and 5-year survival rates for liver transplant recipients are 82.5%, 73.5%, and 67.5% respectively (24). The well-known causes of death during the first year after transplantation include graft dysfunction, technical problems, and infections (25). There have been several studies looking at the cause of death in patients who have survived at least 1 year. These studies have generally defined the patient with survival greater than 1 year as a long-term

survivor (26,27). A few studies have looked at mortality after 3 or 5 years (28,29,30,31). The common evolving theme is that mortality occurring more than 1 year after liver transplantation in adults includes malignancy (recurrent and de novo), recurrence disease (especially hepatitis C virus [HCV] infection), cardiovascular events, chronic rejection, and less frequently infections and chronic renal failure (CRF). Table 56.1 illustrates the common causes of death in patients having survival greater than 1 year.

Table 56.1. Long-Term Causes of Death Post–Liver T

Author site	Years study	Number of patients died after 1 year	F/U years mean	Patients with malignancy	Recurrent disease	Cardiova
Astar S, et al.	1982–1993	36/494	(1–10)	10 (28%)	3 (8%)	5 (14%)
London, Canada		(7.5%)		HCC 5	HBV 3	MI 3
				Leuk 2		Aneuys 2
				Lymp 1		
				Panc 1		
				Cca 1		
Janin A, et al.	1981–1998	1,240/4,000	9.4	140 (5.4%)	Unknown	91 (3.1%
Pittsburgh		(31%)	(2–18)			
Rabkin J, et al.	1991–2000	40/459	3.4	9 (23%)	8 (20%)	4 (10%)
Oregon		(9.6%)	(1–8.6)	HCC 3	HCV 7	
				Cca 2	PSC 1	
				Lung 2		
				Renal 1		
				PTLD 1		
Neuberger J			5	19%		22%

Birmingham,						
UK						
Sudan D, et al.	Unknown	127/686	5.2	22 (17%)	Unknown	25 (20%
Nebraska						
Pruthi J, et al.	1984–2001	38/399	Unknown	17 (44%)	9 (24%)	8 (21%)
S. Cal and				PTLD 8	HCV 8	MI 6
British Columbia				Lung 3	Etoh 1	CHF 2
				Colon 2		
				Gastric 1		
				Breast 1		
				Adeno 1		
				Leuk 1		
Voght D, et al.	1984–2001	88/433	5.6	15 (17.5%)	27 (30%)	18 (20%
Cleveland					HCV 16	
					HBV 5	
					Etoh 3	
					PSC 2	
					PBC 1	
Moreno Planus JM, et al.	1986–1996	46/183	Unknown	14 (37%)	15 (32%)	3 (6%)
Madrid			>1 y	HCC 5	HCV 12	MI 3

				Cca 2 Gastric 2 Ampul 1 Esoph 1 lymph 1 Prostate 1 Colon 1	HBV 3	

HCC, hepatocellular carcinoma; Leuk, leukemia; Lymph, lymphoma; Panc, pancreas cancer; Cca
myocardial infarction; Aneuys, aneurysm; PTLD, post-transplant lymphoproliferative disorder; P
adenocarcinoma of unknown etiology; HCV, hepatitis C virus; Etoh, alcoholic liver disease; CHF,
cirrhosis; Ampul, ampullary carcinoma; Esoph, esophageal carcinoma.

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Recommendations

- *Recognition, prevention and surveillance for common causes of late mortality are imperative to improve the survival of this patient population*.

Medical Care

Obesity

Obesity is a major health problem in the United States In the NHANES III study, 22.5% of the general public had a body mass index (BMI) of greater than 30 kg/m^2. Weight gain is also common after liver transplantation. In a large cohort study, 21.6% of nonobese patients become obese in the first 2 years following transplantation (32). The most recent study noted that by 1 and 3 years post-transplantation, 24% and 31% of patients had a BMI greater than 30 kg/m^2 (33). Predictive factors included age greater than 50 years and pretransplantation BMI greater than 30. Another study reports development of obesity after transplantation in up to 60% of patients (34). Appetite stimulation caused by corticosteroids, change to a less restrictive diet, lack of exercise, and higher cumulative doses of prednisone lead to increased weight. An early study from Virginia, reported that severely obese patients had greater transfusion requirements, higher wound infections, and higher rate of death due to multiorgan failure (35). Nair et al. in a single-center study from Johns Hopkins University, evaluated 121 patients to compare outcomes in moderate and severely obese recipients versus nonobese recipients. Postoperative complications of respiratory failure (23% vs. 3%) and systemic vascular complications (14% vs. 2%) were much higher in the severely obese group (36). The length of hospitalization and total hospital costs were higher in severely obese patients compared to nonobese patients. The same investigators, using the UNOS database, assessed the morbidity and mortality in obese patients after liver transplantation (37). Primary graft nonfunction and immediate, 1-year, and 2-year mortality were significantly higher in the morbidly obese group. Five-year mortality was higher in the severely obese (28%) and morbidly obese patients (27%) mainly due to infections and cardiovascular events. This study has led many centers to institute weight-restriction guidelines for liver transplantation. Interestingly, no studies exist on the success of weight loss after transplantation, either by diet or by obesity surgery. The National Heart, Lung and Blood Institute and National Institute of Diabetes and Digestive and Kidney Diseases guidelines for obesity recommend a target of 10% of baseline weight at a weight loss rate of 1 to 2 lb/week (38).

Recommendations

- *Yearly evaluation for weight gain*.
- *If obesity develops, target weight loss of 1 to 2 lb/week. This requires multiple clinical visits and the supervision of a nutritionist*.

Gout

Hyperuricemia is a common disorder associated with liver transplantation (39,40). Calcineurin inhibitor immunosuppressant medications impair the excretion of uric acid (41). Neal et al. in a study of 134 consecutive liver transplant recipients, found that 47% of patients had hyperuricemia (39). The levels correlated with renal dysfunction. Interestingly, only 6% developed gout located in the wrist, knees, ankle, or elbow at a mean of 25 months after transplantation. All those treated with allopurinol had resolution of their symptoms. In another study, hyperuricemia was present in 85% of patients, with only 3% developing gout (40). Therefore, with the incidence of gout being quite low, treatment of isolated hyperuremia with allopurinol is not likely needed. The management of gout in these patients is potentially complicated by interactions of immunosuppressants and renal insufficiency. The combined use of azathioprine and allopurinol may lead to myelosuppression. Extreme care should be used with this combination.

Recommendations

- *Allopurinol 100 mg/day, for patients with a history of gout and hyperuricemia*.
- *Acute gout may be treated with colchicine, 0.6 mg every 2 hours up to 5 doses*.
- *If symptoms persist, a trial of prednisone tapered over 1 week may be helpful*.

Vaccines

Immunosuppressed liver transplant recipients are at risk for infections, even many years after transplantation. Influenza poses a yearly risk. Previously published studies reported influenza vaccination response in adult liver transplant recipients to be from 50% to 95% seroconversion (42,43,44). These authors recommended a second dose to increase response rates. Soesman et al. found an increase of 20% in response rate with this strategy (44). Duchini et al. studied 20 post-transplant recipients at baseline and 6 weeks after vaccination (45). Side effects were well tolerated, but all had significantly lower titers than healthy individuals.

In 1996, the Advisory Committee on Immunization Practices recommended all patients with chronic liver disease be vaccinated for hepatitis A virus (HAV).

Unfortunately, some patients are not vaccinated pretransplantation and may require the vaccine after transplantation. In a liver or renal transplant recipient study, Stark et al. found a seroconversion rate of 97% for HAV vaccination, but unfortunately found a rapid decline in antibody titers (46). Furthermore, only 39% of cyclosporine-treated patients had protective antibody titers versus 79% of tacrolimus-treated patients. A Mayo Clinic study of 39 patients evaluated HAV seroconversion rates at 1 and 7 months after the first dose utilizing two standard doses given at 6 months apart (47). Seroconversion rates were dramatically lower in the OLT patients compared to patients with chronic liver disease and healthy controls: 1 month (8% vs. 83% vs. 93%) and 6 months (21% vs. 97% vs. 98%). The few responders were much longer away from transplantation (average 75 months) than nonresponders, which may be related to higher immunosuppression at time of vaccination.

Prevention of hepatitis B after liver transplantation is becoming less difficult with the use of hepatitis B immunoglobulin (HBIG) and nucleoside analog medications. Several investigators have tried hepatitis B vaccination as another alternative (48,49,50). Sanchez-Fueyo et al. in a pilot trial of recombinant hepatitis B virus (HBV) vaccine in liver transplant recipients reported anti-HBs titers greater than 10 IU/L in 14 of 17 patients. However, as described in an editorial, the patients were atypical in being many months removed from transplantation (48). The Berlin group evaluated antibody response to hepatitis B surface antigen in 10

patients 2 years after transplantation (50). In contrast to other studies, patients were continued on HBIG during the study. They additionally received 20 µg of recombinant HBV antigen with a novel adjuvant (monophosphoryl lipid A) at weeks 0, 2, 4, 16, and 18. Five of ten patients developed antibody titers greater than 500 IU/L.

We have previously reported on pneumococcal vaccination before transplantation but to our best knowledge no study has been performed on pneumococcal vaccine response in patient after transplantation (51).

Recommendations

- *Yearly influenza vaccination of liver transplant recipients*.
- *All personnel associated with care of liver transplant recipients should receive the annual influenza vaccination*.
- *Vaccinate before liver transplantation if possible for HAV*.
- *If unvaccinated for HAV before transplantation, consider vaccination, however, response rate will be better after 1 year removed from transplantation*.
- *Discontinuation of passive immunoprophylaxis of HBIG with HBV vaccination has not become common practice. Large multicenter trials need to be performed to address this issue*.

Bone Disease

Osteoporosis is characterized by reduced bone mass and altered architecture increasing the risk of fracture typically in the spine, hip, or wrist areas (52). Many patients may have severe osteoporosis due to their chronic liver disease, especially cholestatic liver diseases. Early studies report immediate bone loss after transplantation with the nadir of bone mineral density (BMD) being around 6 months after transplantation with gradual recovery (53,54,55). Feller et al. noted in 28 patients that spine BMD had returned to pretransplantation levels by a mean of 85 months (56). However, another study did not show any improvement in the BMD of the proximal femur 36 months after transplantation (57). Guichelaar et al. reported gradual improvement of osteoporosis with follow-up to 7 years post-transplantation (58). Hamburg et al. reported that lumbar bone density increased in the second year after transplantation, stabilizing thereafter with follow-up to 15 years (59).

Fractures are common in the high-risk group, most commonly in the hip, pelvis, spine, ribs, and wrist with frequently reported risks ranging from 5% to 35% (60,61). Haagsma et al. in a prospective study of 36 adult patients reported development of vertebral fractures in 38% of patients within 6 months of transplantation (62). A more recent prospective study by Hardinger et al. from St. Louis followed up 153 patients over 10 years (63). The prevalence of symptomatic fractures was 15% at a mean of 2.2 years post-transplantation. The only factor associated with risk of fracture was being female. Age, time from transplantation, race, menopause, renal insufficiency, family history of osteoporosis, BMD, and T score did not predict either osteoporosis or fractures after liver transplantation. Others have found older age (64), vertebral fracture before transplantation (61), cholestatic liver disease (60), and smoking (61) to be risks for fractures.

Immunosuppressive medications are also proposed to contribute to osteoporosis. Glucocorticoids increase bone resorption by inhibition of osteoblast activity even at low doses of less than 7.5 mg per day (65). Cyclosporine has been shown to increase osteoporosis risk in renal transplant recipients (66). A histomorphometric analysis of 33 patients after liver transplantation by the Mayo Clinic suggested that patients with tacrolimus therapy have earlier recovery of bone formation and trabecular structure by 4 months compared to cyclosporine-treated patients (67). The role of secondary hyperparathyroidism remains controversial in this patient population with small increases in parathyroid levels within a few months of transplantation (68).

Vitamin D deficiency is not rare in patients with chronic liver disease and Feller et al. demonstrated a rapid rise in 25-hydroxyvitamin D levels after transplantation (56).

Treatment of osteoporosis after liver transplantation has usually been reported in small uncontrolled series. Velero et al. noted increased BMD in 40 patients treated with calcitonin (69). Whereas, Hay et al. found subcutaneous calcitonin of 100 IU/day ineffective in preventing fractures in a subset of patients undergoing transplantation for primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) (70). Neuhaus et al. found improvement in BMD with daily doses of calcitriol, calcium, and sodium flouride (71). Two studies report on the use of IV pamidronate but showed little efficacy in preventing fractures (72,73). Interestingly, no data for oral bisphosphonates has been reported.

Two comprehensive reviews by Crippin and Compston provide excellent guidelines on management of osteoporosis in liver transplant recipients (74,75).

Recommendations

- *Pretransplantation evaluation with dual energy x-ray absorptiometry (DEXA) bone scan, serum tests of calcium, phosphorous, parathyroid hormone level, testosterone (men), estradiol (women), and a 25-hydroxyvitamin D levels*.
- *Treatment of all patients with 1,500 mg of calcium/day and correction of any deficiencies*.
- *If severe osteoporosis is noted by a T score greater than 2 SD below normal, then consider starting oral bisphosphonates*.
- *Those under treatment for osteoporosis should have yearly DEXA, measurement of calcium, phosphorous, and thyroid function*.

Cardiovascular Complications

Established risk factors for development of cardiovascular disease (CVD) in liver transplant recipients include hypertension, hyperlipidemia, hyperhomocysteinemia, diabetes, renal insufficiency, obesity, family history of CVD, advanced age, African American race, male gender, tobacco abuse, and immunosuppressive medications (76,77,78,79,80,81) (Table 56.2). Remarkably, the extent of morbidity and mortality related to CVD remains controversial (79). In an early study from the United Kingdom, 110 consecutive patients were studied, the relative risk for cardiovascular deaths was two and half times greater as compared to age-matched population without transplantation using the Framingham risk score (77).In contrast, a Spanish study, found that the prevalence of hypertension, diabetes, hypercholesterolemia, and hypertriglyceridemia was lower at 5 years after transplantation compared to 1 and 3 years after transplantation and no different from the general Spanish population (80). Neal et al. in a comprehensive study of cardiovascular complications from the United Kingdom looked at the prevalence of risk factors for coronary heart disease and the effect on the predicted 10-year risk of developing coronary heart disease and incidence of cardiovascular events (82). One hundred and eighty-one patients were studied with most of them treated with cyclosporine. The 10-year predicted risk of CVD increased to 11.5% compared to 6.9% before transplantation and matched the control population of 7% at 10 years. Compared to matched controls, the incidence ratios of myocardial infraction and stroke were 0.55 and 1.45. Therefore, by 10 years the mortality associated with CVD was similar to that of the general population.

Hyperlipidemia is common after liver transplantation (Table 56.2). Mixed hyperlipidemia (types 2a, 2b, and 4) with high cholesterol and triglyceride levels is the common pattern after transplantation. The etiology of hyperlipidemia is multifactoral (79). Steroids increase secretion of very low-density lipoprotein (LDL) and conversion of LDL. Cyclosporine inhibits 26-hydroxylase reducing the transport of cholesterol into bile, and binds to LDL receptor increasing the levels of LDL cholesterol (83). Tacrolimus in several studies has been noted to cause a lower incidence of dyslipidemia than cyclosporine (84,85). A study by investigators in Philadelphia, in converting patients from cyclosporine to tacrolimus monotherapy found that mean cholesterol levels decreased from 251 to 202 mg/dL and

triglycerides decreased from 300 to 207 mg/dL (86). Several studies have evaluated dyslipidemia with sirolimus therapy in liver transplantation (87,88,89). In 57 patients treated with a combination of cyclosporine or tacrolimus with sirolimus Trotter et al. found hypercholesterolemia and hypertriglyceridemia were more common in the combination of cyclosporine and sirolimus than tacrolimus (hypercholesterol, 30% vs. 6%, hypertriglycerides, 18% vs. 3%) (87). Whereas in a more recent study of patients receiving sirolimus and tacrolimus for a mean of 57 months after transplantation compared to a group of tacrolimus monotherapy or in combination with mycophenolate mofetil, hypercholesterolemia and hypertriglyceridemia were similar (hypercholesterol, 15% vs. 9%, hypertriglycerides, 10% vs. 9%) (88). The proposed mechanism of sirolimus-induced hyperlipidemia is thought to be related to elevated apoCIII levels which inhibit lipoprotein lipase activity (90). Surprisingly, little data exists on use of statins for hyperlipidemia in liver transplant recipients. In a small study of 16 patients, cerivastatin and pravastatin decreased cholesterol by 21% and 15%, and LDL by 27% and 17% (91). Our center uses atorvastriatin because it is more potent and treats both cholesterol and triglycerides.

Hypertension early after transplantation is almost universal, likely due to high vascular volume and high

levels of calcineurin inhibitors, which reduce endothelial release of vasodilating factors (nitric oxide and prostacyclin) and increase the release of vasoconstrictor factors (endothelin and thromboxane) (92). Neal et al. found an increase in plasma endothelin levels and increases in arterial stiffness associated with hypertension (93). Earlier hypertension studies within the first year noted hypertension ranging from 17% to 82% (76,77,78). In a large European study at 6 months after transplantation, 26% of cyclosporine-treated patients and 17% of tacrolimus-treated patients had hypertension (94). More recent studies using a standard definition of hypertension reading greater than 140/85, reported that incidence at 3 months was 82% in cyclosporine patients and 32% in tacrolimus patients, and at 6 months 50% of patients were hypertensive (93). In studies with longer follow-up from 3 to 5 years after transplantation, overall incidence was around 50%. Detailed analysis in the Rabkin et al. study noted that 58% required a single antihypertensive agent, 29% required two agents, and 10% needed three agents (95). Galioto et al. found that nifedipine was effective in only 22% of patients, whereas 33% of carvedilol treated patients after liver transplantation responded (96). In a large study, Neal et al. reported that 46% of patients responded to amlodipine, with a decrease in systolic blood pressure from 154 mm Hg to 130 mm Hg (97). Patients unresponsive or intolerant to amlodipine were randomized to bioprolol or lisinopril. Lisinopril was successful in 84% of patients, reducing the systolic blood pressure from 154 mm Hg to 130 mm Hg.

Table 56.2. Cardiovascular Risk

Author	Number of patients	F/U time from orthotopic liver transplantation	Percentage with hypertension (%)	Percentage with hypercholesterolemia (%)	Perc hypert
Varo E, et al.	184	4.2 y	82 csa	15	Unkno
Spain 2002			32 fk		
Fernandez-Maranda C, et al.	116	5 y	49	34	11

Spain 2002					
Rabkin J, et al.	87	3 y	62 csa	10	Unkno
Oregon 2002			38 fk		
Johnson S, et al.	110	3.9 y	63	44	Unkno
London 2002					
Neal D, et al.	181	10 y	83 csa	59	41
Cambridge 2004			67 fk		

Csa, cyclosporine; fk, tacrolimus.

Recommendations

- *Monitor fasting lipid profiles at 6 months and 1 year post-transplantation, then annually*.
- *Attempt to attain total cholesterol levels less than 200 mg/dL, LDL levels less than 130 mg/dL, and normal triglycerides levels after transplantation*.
- *First-line treatment should be dietary modification in accordance with the American Heart Diet*.
- *If unsuccessful, 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are to be used*.
- *Early treatment of hypertension incorporates the use of diuretics, clonidine, or calcium channel blockers*.
- *Diltiazem and verapamil can alter cyclosporine or tacrolimus levels, therefore are rarely used*.
- *Angiotensin-converting enzyme inhibitors especially in patients with diabetes are a good choice for treatment of hypertension*.

Diabetes

The prevalence of diabetes in candidates for liver transplantation has ranged from 4% to 13% in previous studies (98,99). In a prospective study of 115 liver transplantation candidates, a group from Spain describes 25% of patients having impaired glucose tolerance and 53% as having diabetes by oral glucose tolerance test (100). Therefore, many patients are at risk of worsening diabetes after transplantation. Immunosuppressive medications are diabetogenic. Corticosteroids induce insulin resistance. Calcineurin inhibitors decrease insulin synthesis and secretion and induce insulin resistance (101). Cyclosporine and tacrolimus appear to have the same diabetic risk, although some have noted a higher risk

with tacrolimus (102,103,104). Other immunosuppression medications including azathioprine, myophenolate mofetil, and sirolimus do not induce diabetes.

Several risk factors for development of new-onset diabetes after transplantation have been recently characterized. A family history of diabetes among first degree relatives and those of Hispanic or African American ethnicity indicate a greater risk (105,106). Age above 40 years, obesity, and many episodes of steroid resistant rejections are also thought to be risks (106,107,108,109). In a large Canadian study, new-onset diabetes was associated only with transplantation for hepatitis C (odds ratio [OR] 4.12) (110). In the multicenter NIDDK-Liver Transplant Database, hepatitis C was also found to be predictive of transient diabetes, but not persistent diabetes after liver transplantation (109). In a small French study, alcoholic cirrhosis and male gender were independent predictors of new-onset diabetes (107).

The incidence (4% to 38%) of new-onset diabetes after liver transplantation has been variously reported because of terms of definition, duration of follow-up, and changing criteria of diabetes. John and Thuluvath from Johns Hopkins University found that 10.5% of post–transplantation patients developed new-onset diabetes, of whom 18 were insulin dependent and 28 were treated by oral hypoglycemic medications (111). In another large single-center study, 37.7% of patients developed new-onset diabetes, 28% had transient diabetes and 9% had persistent diabetes (98). Using the UNOS database, Yoo et al. that found 7.6% were patients with type 1 diabetes and another 7.6% were patients with type 2 diabetes at 1 year after transplantation (112).

Morbidity has been reported as similar among nondiabetic and diabetic patients (113). However, Khalili et al. reported that the overall infectious episodes in the presence of diabetes were fivefold higher (109). Higher morbidity in diabetic patients versus nondiabetic patients of cardiac episodes (48% vs. 24%), major infections (41% vs. 25%), minor infections (28% vs. 5%), neurologic (22% vs. 9%), and neuropsychiatric (22% vs. 6%) episodes were seen in the Baltimore study (111). Two studies found that survival was not worse in diabetic patients (100,114). In contrast, the UNOS

database study reported that 5-year patient survival was lower in patients with type I (insulin dependent diabetes) compared to those without diabetes (63% vs. 75%) (112). Patients with diet-controlled diabetes had a minimally decreased 5-year survival compared to nondiabetic patients. Furthermore, patients with type 1 diabetes or coronary artery disease were 40% more likely to die within 5 years of transplantation compared to those without these risk factors. A German study had similar poor 5-year survival in patients with type 1 diabetes (104).

Consensus guidelines on new-onset diabetes after transplantation have been developed on the basis of definitions from the American Diabetes Association, World Health Organization, and American College of Endocrinology (115).

Recommendations

- *Management of patients who develop new-onset diabetes is similar to recommendations of patients with type 2 diabetes*.
- *Those with a Hemoglobin A1C level greater than 6.5% should be started on treatment*.
- *Initial treatment is lifestyle modifications (exercise, weight loss) and education (dietary and natural history)*.
- *If glycemic control is unsuccessful with dietary modification, consideration of oral diabetic agents may be considered*.
- *Insulin should be used if blood glucose levels do not fall below 120 mg/dL before meals or lesser than 160 mg/dL after meals*.
- *Liberal use of endocrinology consultation is recommended for patients with difficult to control diabetes*.

Inflammatory Bowel Disease

Conflicting results have been published on the prevalence and severity of IBD after liver transplantation (116,117,118). Differences may be attributed to variation in immunosuppression and/or maintenance IBD medications, duration of IBD, and length of follow-up. Papatheoddoridis et al. reported an aggressive natural history in those not treated with steroids after transplantation (119). Conversely, others have noted mild symptoms and few flares of disease (116,117). Haagsma et al. in a single-center study, reported that 36% of patients with pre-OLT IBD experienced exacerbations at a median of 1 year after transplantation (120). Additionally, 11% of patients developed de novo IBD, with risks of 4% (3 years), 11% (5 years), and 14% (10 years) after transplantation. Interestingly, none of the patients was continued on IBD medications such as aminosalicylates. Most transplantation centers now continue specific IBD medications along with immunosuppressants. A unique feature of this study was that the IBD-free survival was increased in patients not receiving tacrolimus immunosuppression and in those receiving azathioprine. From a different perspective, Dvorchick from Pittsburgh analyzed 303 patients to look at the risk of colectomy after transplantation (121). Twenty-two patients (7%) had colectomy due to refractory disease in follow-up to 12 years post-transplantation. Surprisingly, only OLT was the significant risk factor for colectomy (HR 3.1).

Patients with PSC and IBD may have an increased risk of developing colorectal cancer after liver transplantation (122,123). Prior studies have shown the cumulative risk to be between 9% and 14%. Vera et al. from Birmingham, England studied 82 patients with PSC and IBD to identify risk factors for colorectal cancer (124). Colorectal cancer developed in 9.6% of these patients with a mean interval of 46 months (21 to 68 months) between liver transplantation and cancer onset. The cumulative risk of developing colorectal cancer was 14% and 17% after 5 and 10 years after transplantation. Multivariate analysis identified three variables significantly related to colorectal cancer–dysplasia post-transplantation, duration of colitis more than 10 years, and pancolitis. When all three variables were present the risk of colorectal cancer was 100% by 5 years post-transplantation.

Recommendations

- *Maintenance use of IBD medications after transplantation (i.e., aminosalicylates)*.
- *Yearly colonoscopy with surveillance biopsies in patients who underwent transplantation for PSC*.
- *If dysplasia is found, colectomy is warranted*.

Pregnancy

Recovery of normal menstrual function occurs rapidly (within 1 to 2 months of transplantation) in women (125). Unintended pregnancy can occur, therefore contraception should be a topic discussed immediately after transplantation. The most frequent contraceptive practices noted in a questionnaire were barrier methods and tubal ligation (126). In addition, 70% of sexually active patients indicated satisfaction with their relationship, and 75% had weekly intercourse.

Pregnancy outcomes after liver transplantation have been reported from single centers to national transplantation registries (127,128,129). All reports demonstrate favorable maternal and neonatal outcomes, with low incidence of congenital malformations. The two most recent reports support the results of prior studies (128,129). The mean birth weights were lower than normal

(approximately 50%), at a mean gestational age of 36 weeks with around 50% delivered by cesarean section. The Mount Sinai study noted higher pregnancy complications of diabetes mellitus (37%), anemia (33%), elevated creatinine greater than 1.3 mg/dL (25%), preeclampsia (21%), and hypertension (21%). Elevated liver enzymes, graft loss (2%), and rejection were uncommon in the Pittsburgh study, but the Mount Sinai report describes that 10% of patients had spontaneous first-trimester abortions and 26% had terminations of

pregnancy due to elevated liver function that was thought related to graft rejection. This was more common in patients in whom the etiologies of liver disease were autoimmune hepatitis (4/8) and PBC (2/3).

Table 56.3. Pregnancy Categories for Transplantation Medications

Category B (no evidence of risk)
Prednisone
Category C (risks cannot be ruled out)
Cyclosporine
Tacrolimus
Mycophenolate mofetil
Rapamycin
Lamivudine
Ganciclovir
Interferon
Category D (evidence of risk)
Azathioprine
Category X (contraindicated)
Ribavirin

Common transplantation medications carry pregnancy categories of B to X. (Table 56.3)

Recommendations

- *Counsel regarding contraceptive practice immediately after transplantation*.
- *The National Transplantation Pregnancy Registry advises female liver transplant recipients to wait for more than 1 year to become pregnant*.
- *Pregnancy management should include an obstetrician skilled in high-risk pregnancy and the multidiscipline transplantation team*.

Transplantation-Related Complications

Biliary Complications

After OLT, biliary complications occur in 7% to 50% as described in earlier studies (130,131,132,133,134,135). Previously, biliary complications usually required surgical revision; however, endoscopic or interventional radiologic treatment is now the standard of care. Two recognized categories of biliary complications are strictures (anastomotic and nonanastomotic) and leaks (T-tube or anastomotic). In addition, stricture or leaks can be classified as early (<30 days) or late and may be associated with hepatic artery thrombosis. Leaks may be associated with T-tube migration or after T-tube is removed or poor healing of the duct to duct anastomosis. The aim of therapy of leaks is to divert the bile away from the leak site by decompressing the biliary tree and to allow healing of the leak site. Living-related transplantation has been reported to have biliary complications twice as frequently as OLT due to its challenging technical reconstruction of either a Roux-en-Y hepaticojejunostomy or duct to duct anastomosis (136,137,138,139,140). Furthermore, Halme et al. demonstrated that biliary complications were higher among recipients with cytomegalovirus antigenemia, compared with recipients without (75% vs. 49%) (141). Diagnosis of the biliary complication may be obtained by endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiogram (PTC) or more recently magnetic resonance cholangiopancreatography (MRCP). The Barcelona group verified in a group of 63 patients that MRCP had the correct diagnosis of biliary complication in 91% of patients with a sensitivity of 95% and positive predictive value of 97% (142). However, with a high incidence of abnormal findings many physicians proceed directly to

ERCP or PTC due to the ability to perform therapeutic procedures.

Tables 56.4, 56.5, and 56.6 illustrate the most recent published data on biliary strictures, leaks, and living-related biliary complications. As documented in Table 56.4 overall stricture rates of 4% to 13% are reported, more commonly anastomotic and treated with dilatation and stents. A study from the expert endoscopists of Indiana reported that 24.5% of patients had a biliary complication after OLT, from 1988 to 1999 (143). Strictures and stones were commonly found, which required multiple ERCPs (approximately 3) to successfully treat the complication, with the highest reported outcome of 100% success. In contrast, the Edinburgh group had to convert to a Roux-en-Y hepaticojejunostomy in 60% of patients, most commonly in those with late complications (144). Leaks were equally common (4 to 11%), with anastomotic or T-tube leaks variably noted (Table 56.5). Biliary stents were almost universally used with successful outcomes of more than 85%. However, Wolfsen et al. in a study of only 9 patients demonstrated that sphincterotomy alone was associated with good outcomes in 78% of the patients (145).

Living-related transplantation continues to regularly note biliary complications of 35% to 56% in recipients (Table 56.6). Treatment is more difficult and usually requires a multidiscipline approach. Gondolesk et al. from Mount Sinai provide the most in-depth study to date (139). Forty percent of the recipients had biliary complications equally distributed between leaks and

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strictures regardless of the type of biliary reconstruction. Biliary leaks occurred early (mean 12 days) and were from the anastomosis 62% or cut edge 38%. Strictures were diagnosed much later (mean 277 days) with 36% requiring surgical correction. Patient survival was lower in those who had a biliary complication compared to those who didn't (76% vs. 89%) at 2 years after transplantation.

Table 56.4. Biliary Strictures

Author	Overall percentage (%)	Time from orthotopic liver transplantation (days)	Location (%)	Treatment (%)	Mean number o endoscopic retrog cholangiopancreato
Pfau P, et al.	5	105	Ana 61	Stent 77	3
Penn 2000			Non ana	Dilatation 23	3
n = 13			38		
Chahin N, et al.	4.6	Unknown	Ana 100	Stent 100	2
Milan 2001					
n = 22					

Rerknimitr R, et al.	13	240	Ana 78	Dil + stent	3.8
Indiana 2002			Non ana	100	
n = 55			22		
Thuluvath P, et al.	6	Unknown	Ana 76	Dil + stent	3.4
Baltimore 2003			Non ana	100	
n = 25			24		
Morelli J, et al.	8	53	Ana 100	Stent 96	3
S. Carolina 2003				Dil 4	
n = 25					
Thethy S, et al.	10	160	Ana 88	Stent	Unknown
Edinburgh 2004			Non ana	100	
n = 30			12		

Ana, anastomosis; Non ana, non-anastomosis; Dil, dilatation.

Table 56.5. Bilary Leaks

Author	Overall percentage (%)	Time from orthotopic liver transplantation (days)	Number of patients	Location (%)	Treatment (%)	Mea endosc cholangio
Pfau P, et al.	11	84	31	T-tube 74	Stent 100	

Penn 2000				Ana 26		
Morelli M, et al. S. Carolina 2001	8	43	26	T-tube 42 Ana 42 Other 16	Stent 100	
Rerknimitr R, et al.	6	70	12	T-tube 60	Stent 63	
Indiana 2002				Ana 31	ES 23	
				Other 9	NB 9	
Thuluvath P, et al.	4	Unknown	19	T-tube 53	Stent 100	
Baltimore 2003				Ana 26		
				Other 21		
Thethy S, et al.	7	290	28	T-tube 14	Stent 21	
Edinburgh 2004				Ana 78		

Ana, anstomosis; ES, endoscopic sphincterotomy; NB, nasobiliary tube.

Table 56.6. Living-Related Transplantation Biliary Complications

Author	Overall percentage of biliary complication	Number of patients (%)	Leaks (%)	Stricture (%)	Treatment, number of procedures
Kawachi S, et al. Tokyo 2002	40	20	25	20	Drainage 3 PTC 5 ERC 2
Fondevila C, et	56	46	24	33	Surgery 3

al. UCLA/Barcelona 2003					Dilatation 12
Hisatsune H, et al. Kyoto 2003	36	26	Unknown	36	ERC 15 PTC 4 Surgery 7
Gondolesi G, et al. Mount Sinai 2004	40	39	48	51	Drainage 5 Surgery 18 ERC 2 PTC 7
Shah J, et al. Penn 2004	47	19	55	45	ERC 12

PTC, percutaneous transhepatic cholangiogram; ERC, endoscopic retrograde cholangiogram.

Recommendations

- *Diagnosis of the biliary complication may be obtained by ERCP, PTC, or MRCP.*
- *Biliary strictures are treated with dilatation and placement of progressively larger stents with longer duration (>6 months).*
- *Biliary leaks are treated with biliary stents (usually < 6 weeks duration).*
- *Living-related biliary complications require a multidiscipline team approach.*

De Novo Malignancy

As noted in the prior section of late deaths, development of de novo malignancies in liver transplant recipients has emerged as a severe long-term complication. Incidence rates are reported from 3% to 15% with specific cancer rates 3 to 5 times higher than the general population from large registry data (146,147,148,149,150,151,152). Table 56.7 summarizes the published reports from 2000 to 2005 on de novo malignancy; all are single-center experiences. Earlier studies showed liver transplant recipients have a high rate of lymphoma. These are commonly EBV-driven lymphoma, predominately of B cell origin.

Risk factors for de novo malignancy after liver transplantation include smoking, alcohol use, IBD, and older age. The Pittsburgh experience reported higher risks of oropharyngeal and lung cancers in patients who underwent transplantation for alcoholic cirrhosis (153). The Kings College group showed greater risk of all types of de novo malignancies in patients who underwent transplantation for alcoholic liver disease compared to other etiologies (154). The Cincinnati Transplant Tumor Registry suggests that the incidence of common tumors (such as colon, breast, lung, and prostate) is similar to the general public, but tends to occur earlier (155). A recent study by Oruc et al. of Pittsburgh confirmed that de novo breast cancer incidence was similar to that of age-matched general population (156). The role of immunosuppression influence on de novo malignancy remains uncertain. No difference in de novo tumor development was noted in cyclosporine versus tacrolimus-based immunosuppression in many studies (148,154,157); however, one Pittsburgh study

suggested that the risk was lower with tacrolimus (158). Comparison between individuals administered muromonab CD-3 (OKT3) and those not administered also showed no differences in development of tumors (159). The risk of de novo cancers with newer immunosuppressant medications such as mycophenolate or rapamycin remains unknown.

In a large single-center study, Sanchez et al. noted that basal cell skin cancer developed earlier, 19 months after transplantation (159); whereas lymphoma, breast cancer, lung cancer, and non–basal cell skin cancer developed later (27 to 47 months). The cancers with the longest time from transplantation were head and neck and colon cancers (61 and 50 months).

Several studies have shown marked decreased survival in noncutaneous neoplasia (148,154,157,159). The Pittsburgh group revealed poor 1 year survival in patients who developed oropharyngeal cancer (34%) or lung cancer (37%) (158). Herrero et al. in a recent

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study, reported that 15 of 27 patients died of malignancy at a median of 5 months after diagnosis (157). The Baylor group noted the greatest mortality rates in lung cancer (73%) and lymphoma (69%) (159). An alarming study reported that only 40% of patients had dermatologic or oral examination, or mammograms within 2 years of transplantation (160). Eleven percent had a gynecological examination in follow-up and only 50% had either sigmoidoscopy or colonoscopy.

Table 56.7. Types of De Novo Malignancy A

Author	Number of de novo cancers	Overall percentage	Time from orthotopic liver transplantation months	Lymphoma (%)	Skin cancer (%)	Colon cancer (%)	I s
Haagsma Netherlands, 2001	21	12	Unk	5	57	14	
Jimenez Madrid, 2002	62	12	48	21	25	0	
Sanchez Dallas, 2002	125	9	19–61	28	33	7	
Saigal London, 2002	30	3	45	6	43	3	
Schmilovitz-Weiss Tel Aviv, 2003	8	8	31	12	12	0	
Herrero Paplona,	25	22		44	0	0	

2003							
Herrero Paplona, 2005	63	26	Skin 49 Other 44	11	55	3	

Unk, unknown.

Recommendations

- *Cessation of smoking and alcohol use*.
- *Utilize screening protocols similar to those used in the general population*.
- *Annual examination for skin cancer and use of sunscreen lotion of SP 50 or greater, when exposed to sunlight*.
- *Mammograms and gynecological evaluations should be done biannually for women*.
- *Yearly measurement of prostate-specific antigen in men older than 50 years is recommended*.
- *Patients who undergo transplantation for alcoholic cirrhosis may benefit from annual examination of mouth and throat, combined with chest x-ray*.
- *Patients with IBD must have annual colonoscopy with surveillance biopsies for dysplasia. Colonoscopy every 5 years for liver transplant recipients without IBD is recommended*.

Renal Dysfunction

Development of renal dysfunction is becoming a major problem after liver transplantation in patients with greater long-term survival. Calcineurin inhibitor medications (cyclosporine and tacrolimus) remain the cornerstone of immunosuppression in liver transplantation, however, not without some risk of renal dysfunction. A decrease in glomerular filtration rate of 30% to 50% within 6 months of transplantation is commonly seen (161). Renal biopsies in those with CRF associated the calcineurin inhibitors demonstrate interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and sclerosis and collapse of the glomeruli (162).

The incidence of CRF and end-stage renal disease (ESRD) after liver transplantation has been widely described, likely due to difference in definition, method of measurement, and duration of follow-up (163,164,165,166,167,168). Earlier studies from England in a large cohort group found that by 10 years after transplantation, the cumulative incidence of CRF (serum creatinine [Cr] >250 μM) was 8% and ESRD was 4.2% (169). The Pittsburgh group at 5 years post-transplantation reported an incidence of CRF (Cr >2 mg/dL) of 28% (170). The most frequently sited study by Gonwa et al. from Dallas studied 834 patients with follow-up to 13 years after liver transplantation with a definition of CRF as a Cr of greater than 2.5 mg/dL (171). The incidence of ESRD gradually rose from 1.6% at year 1 to 3% at year 5 and 9.5% at year 13. The total incidence of severe renal dysfunction (CRF + ESRD) at year 13 was 18.1%. A significant difference in development of CRF and ESRD was seen in patients with pretransplantation hepatorenal syndrome compared to those without (by year 13, HRS group: CRF 8%, ESRD 11% vs. non-HRS group: CRF 4%, ESRD 4%). Postoperative 1-year Cr, 3-month Cr and 4-week Cr were predictive of CRF and ESRD. Additionally, a profound difference in survival (27% vs. 71%) was noted by year 6 after transplantation in

patients who developed ESRD receiving hemodialysis compared to those receiving a kidney transplant. The Mayo Clinic has additionally described an incidence of renal dysfunction of 10% at 10 years after transplantation as measured by glomerular filtration rate (GFR) by iothalamate clearance of lesser than 40 mL/minute/body surface area (165). If a patient had a GFR of less than 40 at 1 year, by year 3, 65% still had renal dysfunction. ESRD (dialysis or kidney transplant) developed at a mean time of 7.5 years from transplantation. Similar results from the Baltimore groups of a serum Cr greater than 1.2 mg/dL at any time before transplantation or a baseline GFR less than 70 mL/minute/1.73 m^2 or a GFR of less than 30 mL/minute/1.73 m^2 at 3 months were predictive of renal failure (166). Velidedeoglu et al. from Philadelphia analyzing 181 patients found that pretransplantation diabetes (OR 5.7) and early postoperative acute renal failure (Cr >2 mg/dL, OR 10.2) were predictive of CRF (164). The most comprehensive study of this topic was reported recently by Ojo et al. using the Scientific Registry of Transplant Recipients database from January 1990 to December 2000 analyzing patients who received either a heart, lung, heart–lung, liver or intestine transplant (172). With a median follow-up of 36 months, CRF developed in 16.5% of patients, of which 29% required dialysis or renal transplantation. Risk factors associated with CRF included advanced age, female sex, pretransplantation hepatitis C infection, hypertension, diabetes mellitus and postoperative acute renal failure. Those that developed CRF had a fivefold greater risk of death compared to those without CRF. Concentrating on the liver transplantation recipients, by year 5 after transplantation 18% developed CRF, a higher rate than all other types of transplants except intestinal transplantation. This study concludes that an enormous financial burden of treatment of ESRD in this population is likely in the future, if not already here.

What can be done to reduce this risk of CRF in liver transplant recipients? First, can immunosuppression be altered? Several studies have tried substitution of calcineurin inhibitors with mycophenolate moretil (173,174,175,176,177)

or rapamycin (178,179,180) with mixed results and complications (Table 56.8). Poorer results are noted when exchange is long after transplantation. Furthermore, the largest change in GFR is in the first 6 months, leading many to consider delayed use of calcineurin inhibitors.

Table 56.8. Renal Dysfunction

Authors	Number of patients	Conversion time from orthotopic liver transplantation (months)	Conversion type	Renal dysfunction definition	Renal outcome	C
Barkmann et al. Hannover, 2000	22	30	CNI→MMF	Cr >125 µmol/L	201 µmol/L→167 µmol/L	
Schlitt et al.	14	Unknown	10 pt csa→MMF	Cr >125 µmol/L	168 µmol/L→123 µmol/L	
Hannover, 2001			4 pt fk→MMF			

Neau-Cransac et al.	8	85	7 pt csa→MMF	Cr >200 µmol/L	269 µmol/L→220 µmol/L	
Bordeaux Cedex, 2002			1 pt fk→MMF			
Hodge et al.	11	Unknown	6 pt csa→MMF	Cr >1.8 mg/dL	Csa pts GFR 35 mL/min→57	
Philadelphia 2002			5 pt fk→MMF		FK pt GFR 55 mL/min→57	
Raimondo et al.	12	45	csa→MMF	Cr >120 µmol/L	179 µmol/L→<120 µmol/L	
London, 2003						
Cotterell et al. Richmond 2002	7	72	CNI→rapa	Cr >2 mg/dL	2.4 mg/dL→ 1.5 mg/dL	
Nair et al.	16	96	9 pt csa→rapa	CrCl 20–70 mL/min	Cr 1.9 mg/dL→1.5 mg/dL	
New Orleans 2003			7 pt fk→rapa			
Do et al. D etroit 2004	28	26	CNI→rapa	Cr >1.8 mg/dL	Cr 2 mg/dL→2.8 mg/dL	

CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; Cr, serum creatinine; csa, cyclosporin tacrolimus; GFR, glomerular filtration rate; rapa, rapamycin; CrCl, creatinine clearance; ESRD, renal disease.

Recommendations

- *Those with known variables of high risk of development of CRF should be considered for nontraditional immunosuppression and careful monitoring*
- *Consider calcineurin inhibitor free immunosuppression regimen for patients with*

development of renal insufficiency

- *If progressive renal dysfunction is seen, evaluation by ultrasound and consultation by nephrology is warranted*
- *Kidney transplantation offers such a significant survival difference compared to chronic dialysis, therefore patients should be counseled regarding these differences*

In summary, with longer survival of patients after transplantation more frequent complications related to medical, immunosuppression and surgical treatment of these patients is observed. However, the QOL appears to improve in the long-term survivor. Common complications of development of de novo malignancy, CVD or diabetes require annual evaluations and surveillance to prevent or lower the risk of morbidity or mortality. Renal dysfunction appears to be increasing with often dire consequences. Lastly, recurrent hepatitis C is becoming the most pressing and overwhelming problem with a low chance of eradication with treatment after transplantation.

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Chapter 57
The Surgical Options of Liver Transplantation

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Key Concepts

- Liver transplantation has become the victim of its own success; recipient need far exceeds donor supply.
- Donation after cardiac death (DCD) is becoming an accepted alternative technique to increase the size of the donor pool.
- Split-liver transplantation provides transplants for two recipients from one cadaveric organ. This is either performed as an extended right lobe graft for an adult recipient and a left lateral segment graft for a pediatric recipient or alternatively as right and left lobe grafts for two adult recipients.
- Living donor liver transplantation (LDLT) for adult recipients has also become an established means of increasing the number of organs available for transplantation. In the United States of America, centers have been slow to adopt this technique primarily over concerns for donor safety.
- There are unique issues to right lobe living donor transplantation with respect to graft size, venous outflow and biliary complications that make this a particularly challenging operation.

Liver transplantation is the only effective treatment available for patients with severe complications of end-stage liver disease. Unfortunately, the successful application of the treatment has led to increasing referrals that far exceed the cadaveric donor supply. Over the last decade the number of people on the liver transplant waiting list has increased dramatically with 17,913 registrants based on UNOS Scientific Registry Data as of August 2005 (http://www.unos.org). Unfortunately, the number of cadaveric donor livers available for transplantation has increased marginally from 1994 (3,591) to 2004 (5,845). The disparity between the number of potential recipients and the number of available cadaveric donors has led to an inevitable increase in the number of deaths on the waiting list. In 1995, there were 847deaths in patients waiting for liver transplantation compared to more than 1,800 per year in each year since 1999. In this chapter, we will review the standard techniques followed for cadaveric whole liver transplantation as well as the currently used alternatives to increase the number of transplantable livers including split-liver transplantation and living donor liver transplantation (LDLT).

Cadaveric Donor Hepatectomy

Most cadaveric donors are multiorgan donors where the liver is removed along with other abdominal organs such as the kidneys, pancreas and possibly the intestine. The techniques for removal have been somewhat standardized so that the blood supply to each organ is protected. The selection of donors is beyond the scope of this chapter, but generally includes demonstration of normal or adequate graft function by laboratory and/or histologic assessment and an evaluation for the potential transmission of infectious diseases or malignancy. Organs are then allocated according to the UNOS system. For liver allocation, the patient's Model for End-Stage Liver Disease (MELD) or Pediatric End-Stage Liver Disease

(PELD) scores are analyzed by

the UNOS computer and the patient with the highest score is offered the organ.

In most cases (>90% in the United States) organ procurement is from the cadaveric donor in whom brain death has been confirmed and documented according to requirements of the individual state's laws. The donor dissection is performed during normal perfusion of the organs and before the heart has stopped. This allows for careful dissection of the vasculature of each abdominal organ while pulses are palpable. Normally, the aorta and inferior vena cava are completely dissected from the overlying peritoneum, lymph nodes and soft tissues, in order to identify the renal vein and superior mesenteric artery (SMA). The distal aorta is ligated and a very large bore catheter is inserted into the aorta in a retrograde fashion. Next the supraceliac aorta is isolated just below the diaphragm. An additional catheter is sometimes placed (if size permits) into the inferior mesenteric vein for direct portal venous flush. Further dissection of the hepatic hilum is performed to carefully divide the common bile duct, and isolate the hepatic artery and portal vein. The gastroduodenal artery is usually ligated at this point, which assists in tracing the common hepatic artery proximal to the level of the splenic artery. Next the inferior vena cava is isolated above and below the liver. When the dissection is complete, the cold preservation solution (either University of Wisconsin or histidine-tryptophan-ketoglutarate [HTK] solution) is flushed through the distal aortic catheter. A clamp is placed at the level of the diaphragm to stop the flow of solution from entering the thoracic organs while perfusing all of the abdominal organs. Finally, the vena cava is divided just above the diaphragm to allow blood and the preservation solution to exit the organs. Some surgeons place slush on the abdominal organs during the flushing in order to assist rapid cooling of the organs. The liver (often en bloc with the pancreas and/or intestine) is removed first by dividing the vena cava above and below the liver, and the aorta above the celiac axis and below the SMA. Usually in a slush filled basin with preservation solution, these organs are separated by dividing the portal vein, splenic artery and aorta between the celiac axis and SMA, taking care to identify any replaced right hepatic arteries that might arise from the SMA. This can be somewhat difficult when excessive soft tissue is present in the donor, which obscures these planes.

Donor after Cardiac Death

Although the earliest cases of human transplantation were performed with organs procured from donors whose hearts had stopped prior to organ procurement, this practice was largely abandoned after the acceptance of brain death criteria. However, in recent years with the increased need for cadaveric organ donors, attempts to increase the number of available organs has led to the increased use of donors who do not meet brain death criteria. Specific protocols have been established to ensure that the patient is pronounced dead (i.e., lack of cardiorespiratory activity for a specified period of time) by physicians who are not involved with the procurement of the donor organs in order to protect the public from potential abuses. There is an increased risk fof ischemic injury to these organs and only young donors are usually accepted. For the liver allograft, there is an increased risk for both primary nonfunction, which is rare, and for biliary stricture formation, which is more common. In 1998 donor after cardiac death (DCD) donors accounted for 0.7% of the deceased liver donors in the United States. In 2003 this figure has increased to 2.8% and is expected to continue to increase over the next few years (http://www.unos.org)

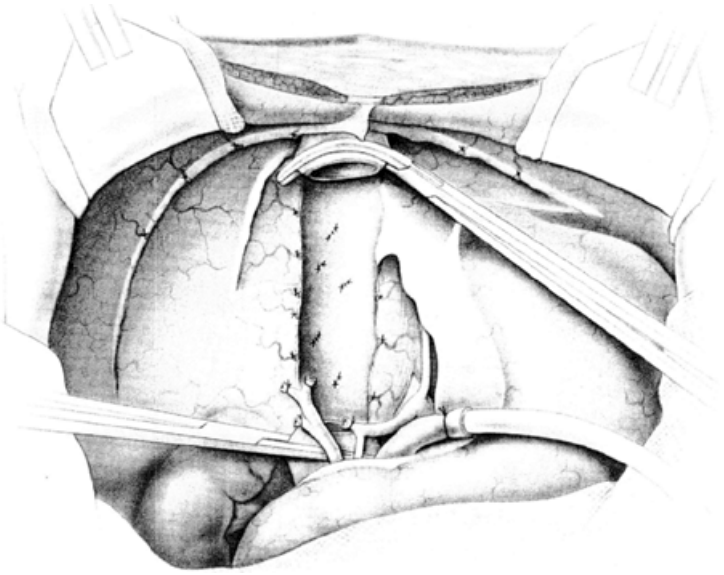
From a technical perspective, in cases where the procurement occurs after the donor's heart beat has stopped, that is, from a DCD donor, skin incision and isolation of vasculature is done rapidly in order to quickly flush with preservation solution (either University of Wisconsin or HTK solution) and rapidly cool the organs. The dissection in DCD donors is then done after cooling the organs and when no blood is flowing through the grafts. Although DCD donors may experience a higher degree of warm ischemia, which can lead to graft injury, the technical aspects of the procedure do not differ other than the speed and order as described in the preceding text.

Liver Transplantation—Recipient Procedure

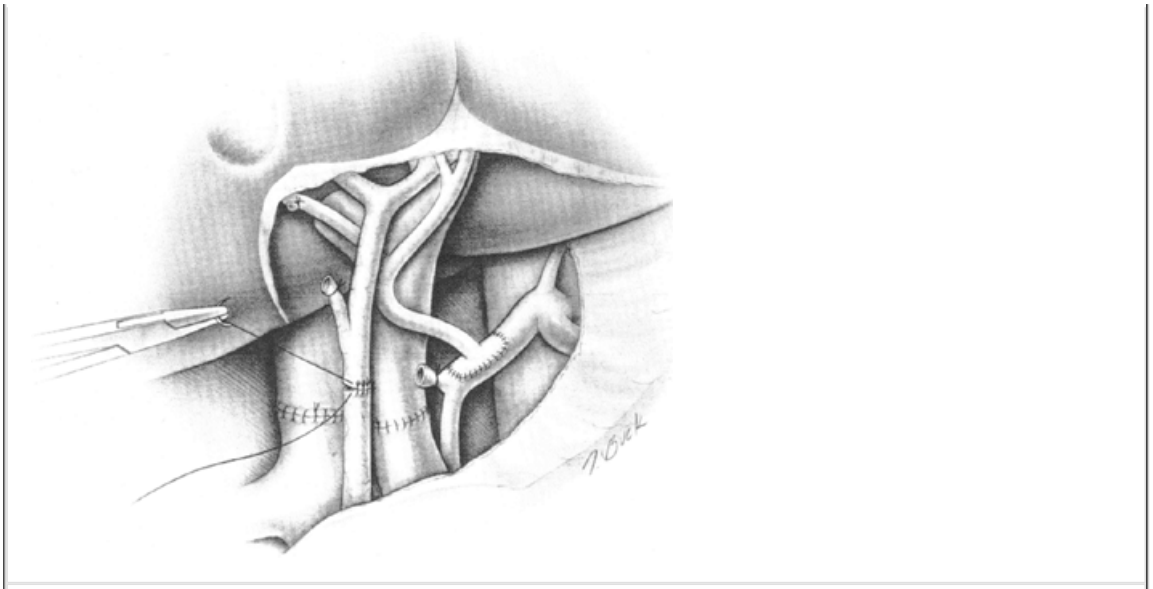
The removal of the native liver at the time of liver transplantation is usually the most challenging part of the procedure and arguably one of the most difficult operations performed by surgeons. The development of portal hypertension and vascularized adhesions places the recipient at high risk for rapid and excessive blood loss. A successful transplantion team not only requires a gifted surgeon, but also requires an experienced team of anesthetists to manage the blood loss and electrolyte shifts intraoperatively. The recipient hepatectomy is achieved by ligating and dividing the native bile duct, the proper hepatic artery or it's individual branches, clamping and dividing the portal vein high in the hilum and dividing the inferior vena cava above and below the liver. Figure 57.1 shows the abdominal cavity after the native hepatectomy.

The whole liver allograft is then taken out of the cold preservation solution. The anastomosis of the donor suprahepatic inferior vena cava is made end to end to the native inferior vena cava at the level of the

native hepatic veins. The infrahepatic vena caval anastomosis is likewise performed end-to-end above the native renal veins in the recipient. The portal venous anastomosis is then performed end-to- end and often the graft is revascularized at this point to minimize the length of warm ischemia. Next the hepatic artery is reconstructed to the native hepatic artery, the gallbladder is then removed and the bile duct is anastomosed to the native bile duct, often over a T-tube. Figure 57.2 shows these attachments in the recipient. There are technical variants to this standard approach such as the piggy-back technique, the use of interposition grafts or the use of the inferior vena cava for portal venous inflow in cases of portal vein thrombosis that are beyond the scope of this chapter.



▪ **Figure 57.1** Abdominal cavity after native hepatectomy.



▪ **Figure 57.2** The recipient's abdomen showing the anastomoses required in the liver transplant procedure.

Alternatives to Standard Cadaveric Liver Transplantation—Historical Perspective

In response to the increasing risk of death for patients awaiting liver transplantation, alternatives to whole organ cadaveric grafts (such as the use of DCD donors described in the preceding text) have been sought. The most commonly used clinical alternative to whole cadaveric liver transplantation has been partial liver transplantation, which includes reduced-size, split-liver and living related transplantations.

Reduced-Size Liver Transplantation

The technique of reduced-size liver transplantation (RSLT) is presented primarily for historic purposes. Although this technique increased the number of livers available for transplantation in children, it had the undesired effect of reducing the pool of cadaveric organs available for adult recipients, who represented more than 90% of the patients on the waiting list. It has therefore been all but abandoned for the alternative of split-liver transplantation described in the subsequent text. RSLT implies the use of a portion of the donor liver and was the first form of partial liver transplantation introduced. The technique of reduction of the liver allows livers from larger donors to be used in smaller recipients, who traditionally had higher rates of mortality on the waiting list (1). It was introduced clinically in 1981 and Bismuth first reported the successful transplantation of a reduced-size liver graft in 1984 (2). The technique was primarily used for the smallest children awaiting transplantation and at one time accounted for as many as 75% of transplants performed on children weighing less than 10 kg (3). The main goal of RSLT was to decrease pretransplant mortality and this was achieved. With this experience it became apparent that these reduced-size livers functioned well and not good as full-sized organs with comparable rates of postoperative complications. The successful application of RSLT directed efforts to divide the liver in such a way that not only the pediatric recipient could receive a transplant, but that the larger right side of the liver could be transplanted into another recipient, often a larger child or adult. This technique came to be known as *split-liver transplantation*.

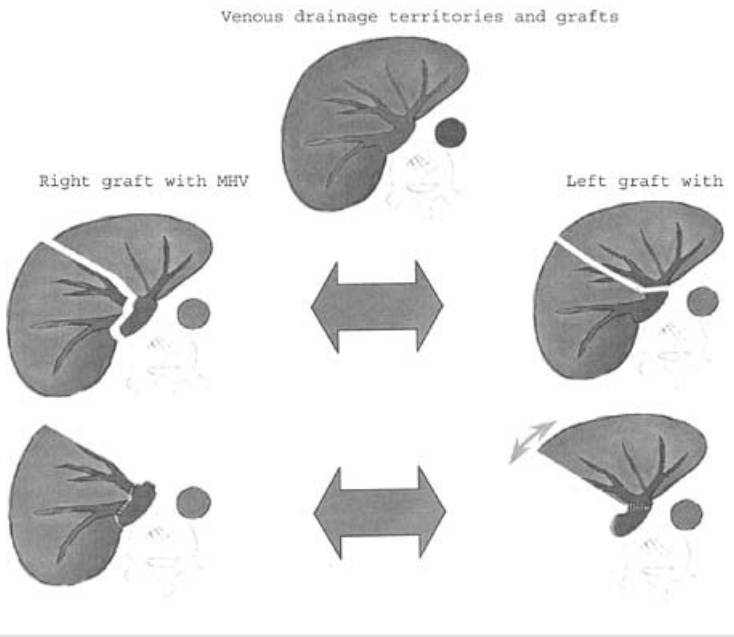
Split-Liver Transplantation

Split-liver transplantation is an attractive concept that provides transplants for two recipients from a single cadaveric liver. Typically, the left lateral segment is

transplanted in a child and the right lobe in an adult (or larger child). The first reported split-liver transplantation was performed by Pichlmayr et al. in 1988 who transplanted the right lobe into an adult with primary biliary cirrhosis, and the left lateral segment into a child with biliary atresia (4). Subsequently, Bismuth et al. reported using the technique to transplant in two patients with fulminant hepatic failure (2). These results were discouraging due to technical complications and poor recipient selection. However, perseverance by a few centers has proved that standard application of split-liver transplantation can increase the number of available liver grafts from cadaveric donors as much as 26% to 28% (5,6). Generally, only optimal cadaveric donors are considered candidates for splitting because of the potential for increased preservation injury, especially if the splitting occurs ex vivo. Estimates based on usual donor characteristics in the United States reveal that between 15% and 25% of cadaveric donors may be suitable for splitting (7).

Ex vivo technique

The technique of ex vivo split begins with a standard whole organ procurement and its packaging in preservation solution. After the graft is transported to the recipient transplantion center, the graft is divided on the back table. The hilar structures (hepatic artery, portal vein and bile duct) are dissected and divided between the two hemi-livers. Cholangiography and/or arteriography to delineate anatomy are performed as needed (5,8). There are varying opinions as to how the vessels should be divided. The key to successful splitting of the liver is to share vascular and biliary structures between the two grafts without compromising either side and preferably providing each graft with a single first-order arterial and biliary structure. Some centers routinely retain the full length of the hepatic artery and portal vein with the left hemi-liver and other centers routinely retain the full length of the vessels with the right hemi-liver. Most centers retain the vena cava with the right lobe graft. Because the left biliary anatomy is more consistent, the left bile duct, which is generally a single duct, is usually divided above the hepatic bifurcation, and the common hepatic duct is retained with the right lobe graft. The parenchymal transection is performed either with the mosquito fracture technique or by sharp dissection. The line of transection extends from the confluence of the left and middle hepatic veins (MHVs) to the right side of the falciform ligament and umbilical fissure down to the hilar plate (Figure 57.3) (9,10). In most centers, Couinaud segments I and IV are resected and discarded, to avoid acute necrosis or less common gradual atrophy (9,11).

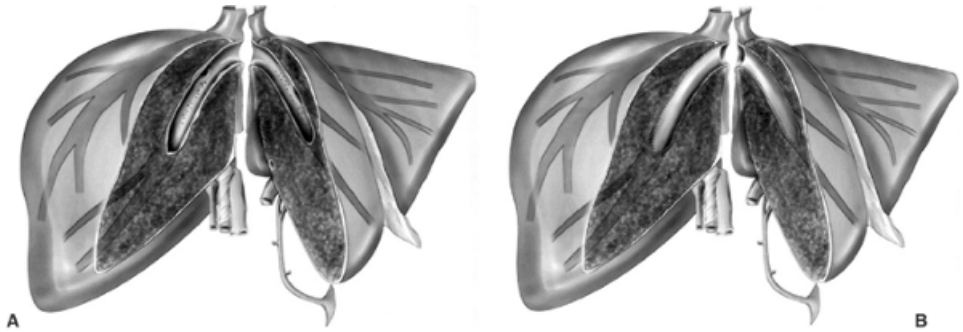


▪ **Figure 57.3** Lines of transection for the different split-liver allografts.

Recently, full-left full-right splitting for two adult recipients has been reported by Colledan et al. This technique leaves a right lobe graft comprising Couinaud segments V—VIII, and a left lobe graft consisting of segments I–IV. The vena cava and MHV are retained with the left lobe graft (12). Early experience suggested that using this technique put the right lobe graft at risk for congestion of the anterior sector (segments V–VIII) and that the recipients of the left lobe grafts may develop inferior outcomes due to small-for-size syndrome. Solutions to these problems include preserving the MHV with the right lobe graft or reconstructing the segment V and VIII veins. Preserving the MHV is a less attractive option as this further reduces the size of an already small left lobe graft. A recent report by Broering et al. describes a novel technique of splitting the MHV between the left and right lobe grafts and using a split iliac vein conduit as a patch venoplasty (Figures 57.4A and 57.4B). This technique in conjunction with their previous description of splitting the vena cava between both grafts creates a common venous outflow for the whole left or right lobe graft. This technique can be performed only by ex situ splitting, but appears to provide improved outcome compared to prior reports of splitting cadaveric livers for two adult recipients (13).

In situ technique

The in situ split technique is essentially the same as the living liver donor technique further discussed in the subsequent text. Rogiers et al. reported the first case of split-liver transplantation with the in situ technique in 1995 and a series of 14 split grafts in 1996 (14,15). A left lateral segmentectomy was completed in the heart beating cadaveric donor and flushed on the back table with preservation solution. This was followed by standard multiorgan procurement of the remainder of the intra-abdominal organs with in situ flushing of University of Wisconsin solution as described in the preceding text. Hepatic segments I and IV could therefore be evaluated in the donor prior to the flushing to assess perfusion after ligation of the left sided vessels, and excised only if the appearance suggested that they were compromised (14). Busuttil et al. in his series likewise performed the hepatic transection without vascular interruption, however he flushed both the hepatic segments in situ prior to removal from the cadaveric donor (7).

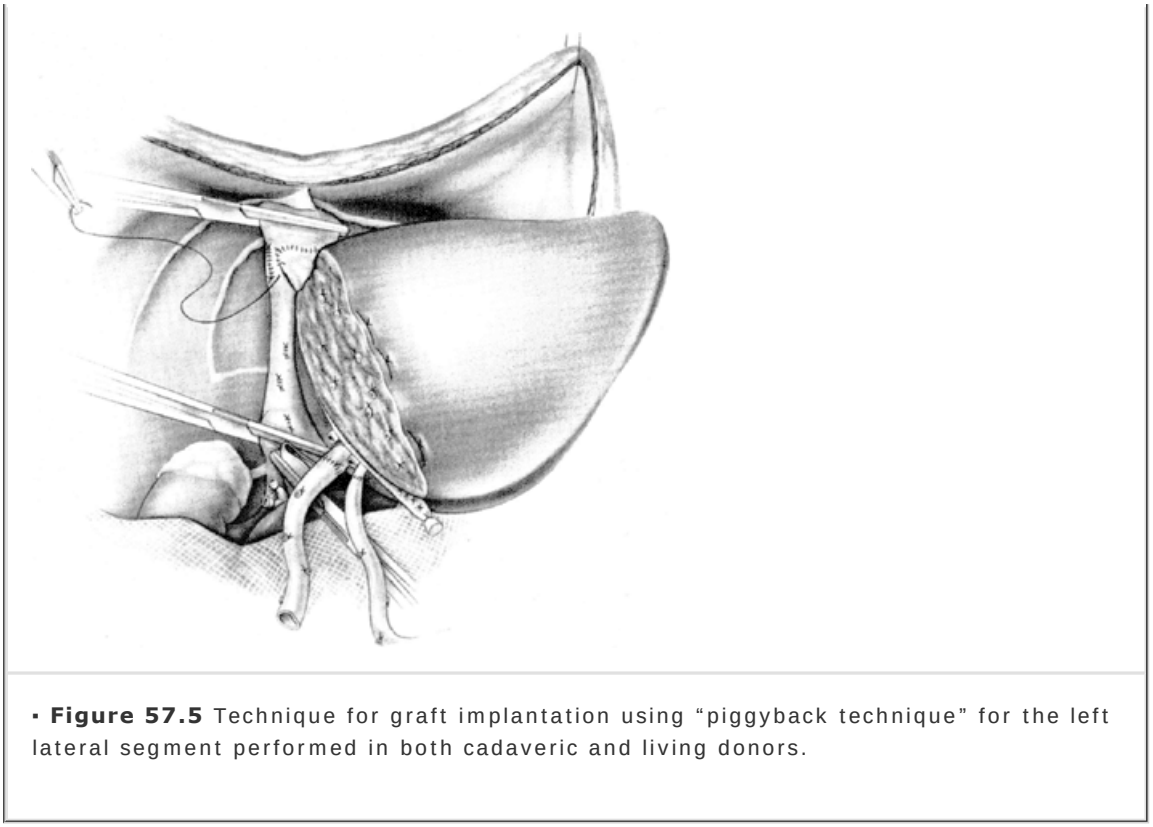


▪ **Figure 57.4** Proposed method for division of the middle hepatic vein for split-liver graft for two adults.

The implantation of the split allograft is independent of the method of procurement (ex vivo or in situ). Generally, the right split graft retains the donor retrohepatic inferior vena cava and is therefore transplanted in the standard orthotopic technique described for whole organ grafts. The left split graft is generally implanted in the so-called piggyback technique to the confluence of the native middle and left hepatic veins (see Figure 57.5). Extension grafts of donor iliac vessels may be used with either the left or right split graft for the hepatic artery or portal vein as needed. Bile drainage is frequently performed by formation of a hepaticojejunostomy, however choledochostomy has also been reported (14).

Results

Until recently, the patient and graft survival rates for recipients of split-liver grafts (50%–67% and 43%–50%, respectively) were lower than the rates for whole organ grafts (6,16,17). The decrease in patient and graft survival was most pronounced for the recipients of the right lobe. The diminished survival was probably a result of poor recipient selection combined with prolonged cold ischemia, and warming of the graft during the ex vivo preparation (7). By restricting split-liver transplantation to elective situations, the results have improved and are now comparable to whole organ grafts even for the right lobe graft (5,18). Reports of in situ splitting have revealed that this technique can be used even in urgent recipients without compromising patient and graft survival (7).



▪ **Figure 57.5** Technique for graft implantation using “piggyback technique” for the left lateral segment performed in both cadaveric and living donors.

Complications of split graft transplantation

Transplantation with a split graft was initially associated with increased bleeding. In the early series as many as one-third of ex vivo split-liver graft recipients required reoperation or transfusion for early postoperative bleeding (6). In contrast, after in situ split-liver grafting, bleeding occurred in less than 3% of recipients (7,14). Ex vivo splitting is also associated with a higher risk of biliary complications (22%–27%) compared to whole organ (4%)or in situ split grafts (0%–3%) (5,6,7,14,18). Rates of arterial thrombosis and primary graft nonfunction in recent series are similar regardless of graft source (7,9,14).

Split-liver grafts more efficiently use cadaveric donors than RSLT because the total number of livers available for transplantation is doubled. Furthermore, with split-liver grafts the redistribution of adult cadaveric livers to pediatric recipients created with reduced-size grafts is avoided (19). Despite the recent publication of splitting for two adult recipients, split-liver transplantation is currently used primarily to provide a left lateral segment graft for a pediatric recipient with an adult recipient receiving the extended right lobe graft. Unfortunately, the most attractive potential application of the technique (i.e., splitting a liver for two adult recipients) has not been generally achieved. There are a variety of reasons for this failure to apply split-liver transplantation for two adults in the United States. One of the most important considerations, however is that the full left lobe of the liver provides inadequate functional hepatic mass for most adult recipients in the United States because of the relative obesity of the population. The acceptable range of hepatic mass required to achieve successful hepatic function after liver transplantation is further discussed in subsequent text. As clinical experience with liver resections and partial liver grafts from cadaveric donors increased, it appeared feasible to safely use living donors for partial liver transplantation.

Living Donor Liver Transplantation

The introduction of live donors for liver transplantation (LDLT) was delayed compared to kidney transplantation. Although LDLT has been practised since the 1960s, and according to UNOS in 2004 accounted for 41% of all kidney transplants performed, LDLT was first introduced in 1989 for pediatric recipients (http://www.unos.org) (20,21). The right lobe

LDLT for adult recipients was introduced much later in 1998 (22). The main impediments to broader application of LDLT to larger recipients were twofold. First, surgeons were concerned with donor safety associated with larger hepatic resections and secondly, they were concerned about providing adequate hepatic mass for the recipient. Owing to the resection of up to 75% of the hepatic parenchyma during a right lobe resection, the risks for complications in the donor are much higher than in left lateral segment liver resection or removal of a kidney. The focus of this section will be a review of world experience with LDLT for adult recipients.

Graft size considerations

Two methods for determining whether a graft will have adequate functional hepatic mass have been developed. The first method involves the calculation of the graft to recipient weight ratio (GRWR) which compares the mass of the graft to the overall body mass of the recipient (23). The second method involves the calculation of the liver volume as a percentage of the standard liver volume (SLV) (% SLV) using a mathematical formula based on measurements of the liver at autopsy (24). Table 57.1 shows examples of both of these calculations. A recent review shows that there is a nearly perfect (i.e., linear) correlation between these two estimates of liver volume, and both the methods can therefore be used interchangeably (23).

Early experience with living donor transplantation in adult recipients

The earliest experience with transplantation of adults using living donors merely extended the pediatric experience using either the left lateral segment or full-left lobe grafts (with or without the MHV) (26,27,28). The size of partial left liver grafts, however, in most cases

corresponds to only 20% to 35% of the recipient's expected SLV (Table 57.1). Although successful graft function has occurred with grafts as small as 0.6% GRWR (25% SLV) (29), others have reported increased risk for postoperative morbidity and mortality with the use of grafts less than 1.0% GRWR (50% SLV) (23,24,30). Grafts less than 0.8% to 1.0% GRWR or “small-for-size grafts” generally demonstrate poor graft function marked by pronounced cholestasis and prolonged coagulopathy (30). The use of left lobe grafts in adult recipients is therefore limited to recipients with body weight in the range of 45 to 55 kg. In the United States, one would expect this weight restriction to represent only a minority of individuals on the waiting list. Indeed, at the University of Nebraska Medical Center this represents only 11% of the adult waiting list. The right lobe of the liver on the other hand represents approximately 60% of the hepatic parenchyma and for most adults in the United States would provide at least 1% GRWR. The Kyoto group performed the first right lobe liver transplant in a child in 1994; however because of concerns about donor safety right lobe living donor liver transplants were not offered to adult recipients until 1997 and 1998 (22,31,32). Since 1998 right lobe liver grafts from living donors have become the standard graft for adult recipients (32,33,34).

Table 57.1. Methods of Evaluating Adequacy of Partial Liver Graft for a Recipient

GRAFT-TO-RECIPIENT WEIGHT RATIO
Example. Recipient body weight = 70 kg
Graft weight = 700 g
GRWR = 700 g ÷ 70 kg = 0.01 (or 1%)

Optimal GRWR = 1%–3%
CALCULATION OF STANDARD LIVER VOLUME [a](SLV) (24)
Japanese formula for SLV (mL) = 706.2 × (body surface area [m^2]) + 2.4
Example. In a 5 ft. 8 in. 75 kg recipient BSA is approximately 1.88 m^2
SLV = 1.33 L (or approximately 1330 g using above formula)
If donor right lobe estimate on computed tomography scan measures 700 g, then 700/1330 = 53% SLV
Optimal % SLV = >50%
SLV, standard liver volume; BSA, body surface area. [a]Since North European and American whites generally larger than Japanese individuals, revised formula in the subsequent text may be more accurate in these populations (25). Heinemann formula (25) for SLV (ml) in whites = 1072.8 × (body surface area [m2])—345.7[a]

Donor Selection

There are two key issues in donor selection. First, living liver donation should be voluntary without coercion and without financial incentives. Secondly, the potential donor should have minor and well-controlled or no medical conditions in order to avoid increased risk from the donor operation. Most living donors have been immediate family members and rarely close friends (34,35). Caution must be exercised in the evaluation process however, because family members may intentionally or unintentionally place pressure on relatives of appropriate age and blood type to donate. The evaluation process includes an interview with the potential donor in the absence of other family members and is ideally performed by a physician who is not a member of the transplant team. The potential donor is informed of the option for the transplant team to provide a “medical excuse” at any time if he/she chooses not to donate. Minors are generally excluded from living donation. Some have raised concerns over the issue of informed consent with the small experience that currently exists (36). The American Society of Transplant Surgeons (ASTS) position paper presented at the May 2000 meeting concurs that insufficient information currently exists “to accurately assign risk for the donor” and this should be acknowledged to the potential donor during the evaluation. The evaluation process of the potential donor includes determination of blood type and confirmation that this is compatible with the recipient's blood type. Other commonly performed laboratory tests include liver and kidney function tests and viral serologies. Most donors in published series are between 24 and 61 years old. Age is not an absolute contraindication to living donation, however older individuals have a higher likelihood of silent cardiac or cerebrovascular disease that may increase the perioperative risk and the liver may have a decreased potential for regeneration in the older donor. Computed tomography (CT) angiography or magnetic resonance (MR) angiography with three-dimensional reconstruction are the current imaging modalities used to delineate hepatic vascular and biliary anatomy and to determine the potential graft and whole liver volume. Magnetic resonance cholangiopancreatography (MRCP) is usually performed when MR angiography is done but intraoperative cholangiography is

usually performed in addition to further delineate biliary anatomy.

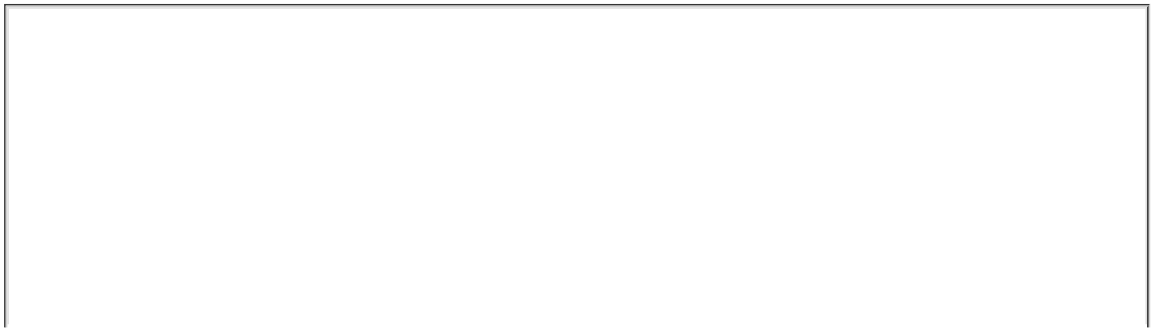
Donor Safety

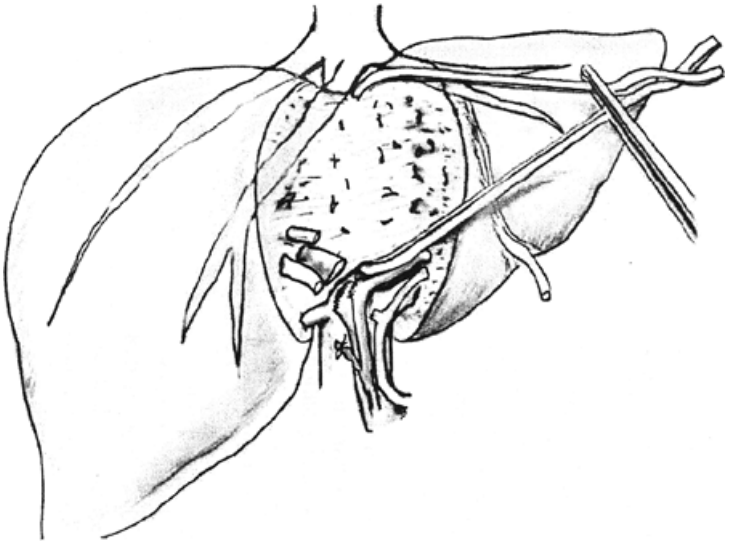
Since the highly publicized donor death in New York, donor safety has become the overriding concern with respect to LDLT. A recent survey by Brown et al. of 449 living donors in the United States documented the actual risk of death to be 0.2% (37). To date however there have been three reported deaths in the United States of America, four in Europe and one each in Japan, India, Egypt and South America (37,38). Interestingly, many of the largest centers in the United States have been the slowest to adopt this technique primarily due to concerns over donor safety. The National Institutes of Health (NIH), the ASTS and the US Department of Health and Human Services have organized a multicenter study to gather and follow sufficient numbers of patients undergoing right lobe LDLT in the United States of America, which will hopefully guide future application of this valuable technique.

Technique for Procurement of the Living Donor Right Lobe Graft

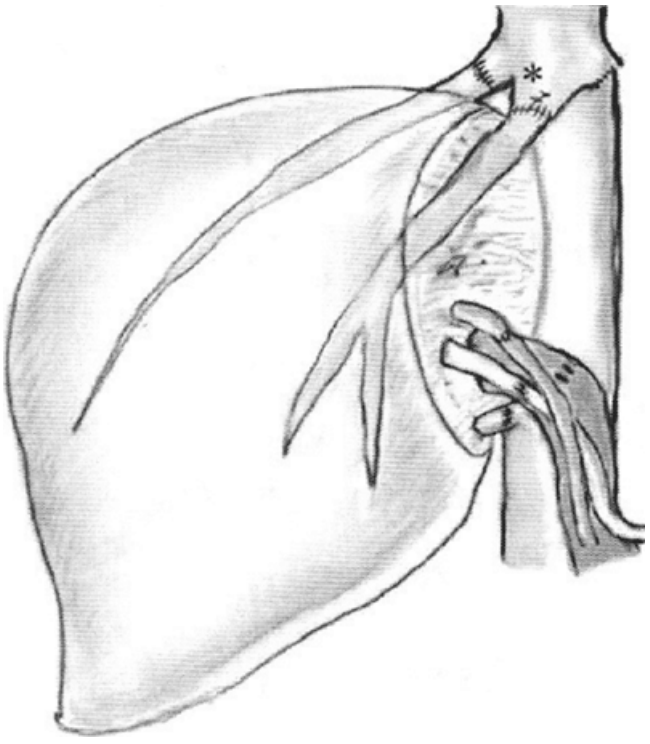
The right liver graft consists of segments V-VIII with or without inclusion of the MHV. There is much debate over the provision of adequate drainage of the anterior sector of the graft and inclusion of the MHV. The donor liver procurement of the right lobe graft begins with a bilateral subcostal incision and division of the hepatic ligaments to the right hepatic lobe. The ligamentous attachments of the left lobe remain intact to prevent torsion of the remnant liver after right lobectomy. The liver is then mobilized off the retrohepatic vena cava carefully ligating small accessory hepatic veins. Marcos et al. recommends preservation of any accessory hepatic vein greater than 5 mm in diameter for reimplantation (33). Intraoperative ultrasound is then used to identify the intrahepatic course of the right and MHVs to determine the optimal line of transection. Hilar dissection is performed to identify the branches to the right lobe from the hepatic artery and portal vein. Minimal if any dissection is performed of the right hepatic duct(s) and left hilar structures. Cholecystectomy and cholangiography are then performed to assess biliary anatomy. The right hepatic vein (RHV) is also isolated prior to parenchymal transection if possible and controlled with a vessel loop. At the University of Nebraska Medical Center the Cavitron ultrasound surgical aspirator (CUSA) ultrasonic dissection is used to transect the hepatic parenchyma. Although this is a somewhat tedious process, it allows identification and ligation of large portal and hepatic venous branches that cross the plane of transection thereby minimizing blood loss. No inflow or outflow vascular occlusion is performed during the process of hepatic parenchymal transection in order to minimize ischemic injury to either portion of the liver. Figure 57.6 shows the right lobe graft after parenchymal transection and division of the hepatic artery, portal vein, right hepatic duct and RHV. Figure 57.7 demonstrates the method of implantation of this

right hepatic lobe graft. This differs from the technique of reduced-size or split-liver right lobe grafts because of the absence of the donor inferior vena cava. In the recipient of the living donor right lobe graft the native inferior vena cava is preserved and the donor RHV is anastomosed directly to the recipient RHV orifice in the so-called “piggy back technique”. Extension vascular grafts are rarely required with the right lobe graft. Biliary drainage is most commonly performed by Roux-en-Y hepaticojejunostomy although duct-to duct anastomosis has also been reported (22).





▪ **Figure 57.6** Technique of living donor right lobe graft including the middle hepatic vein and demonstrating division of the right portal vein, hepatic artery and right lobe bile duct.



▪ **Figure 57.7** Implantation of the right lobe graft.

Ongoing Controversies

The two main controversies with regard to right lobe LDLT that warrant further discussion are the venous drainage of the anterior sector of the right lobe graft and the preferred

mode of biliary reconstruction.

Hepatic Venous Drainage

Although graft size is now well documented enough to be a critical factor in the success of partial liver grafts, the importance of good venous drainage in order to prevent congestion particularly of the anterior sector of the graft and the development of small-for-size syndrome (prolonged cholestasis, coagulopathy and ascites) has been more recently recognized (39). Different approaches have been adopted to prevent congestion from occurring, ranging from reconstruction of segment V and VIII veins to routine inclusion of the MHV with the graft (40). Although Lo et al. routinely include the MHV, the overriding concern with this practice is that the remnant native liver may be decreased excessively in size or have poor venous drainage leading to greater risks for complications in the donor.

Lee et al. reported on five right lobe living donor transplants without drainage of segment V and VIII veins or inclusion of the MHV. They found congestion followed by ascites, cholestasis and sepsis in two of the five, with one of the patients dying twenty days after transplant (41). Subsequently the same authors reported reconstruction of the segment V and VIII veins using interposition vein grafts of either iliac vein or saphenous vein and now recommend this in all cases (42). The Tokyo group proposed a selective approach to drainage of the segment V and VIII veins based on the results of two intraoperative tests during the donor operation (43). (1. After MHV clamping, Doppler flow in the portal vein is assessed. Reversal of flow indicated the need for drainage of the anterior sector veins. 2. After clamping the MHV and the right hepatic artery, if the anterior segment became dusky, drainage was required.) Cryopreserved or autologous vein grafts were used to provide this drainage as needed. Of thirty right lobe living donor transplants performed, 18 required reconstruction based on these tests (42).

The Hong Kong group as noted in the preceding text recommends routine inclusion of the MHV with the right lobe graft in order to avoid congestion of the graft (40,44). They have reported a 96% graft survival at 2 years using this technique. De Villa et al. proposed a selective approach to inclusion of the MHVs only when the right lobe graft volume estimate is <50% SLV (measured by CT scan), the right lobe is drained by a small RHV or large segment V and VIII veins are present (45). Although donor risk is the main concern with this technique, Cattral examined operative risk to the donor in 56 right lobe donors at his center. The outcomes were similar comparing 28 patients where the MHV was included in the graft and 28 patients in whom the MHV was retained in the donor (46). Others have reported prolonged prothrombin times in donors where the MHV was included in the graft; these usually recovered by the end of the first week (47). For now, the issue of anterior sector drainage continues to be controversial with considerable variation in practice from center to center as to whether the MHV is included in the graft or the segment V and VIII veins are reconstructed in the recipient.

Biliary Reconstruction

As mentioned in the preceding text, hepatico-jejunostomy was the standard biliary reconstruction technique for left lateral segment living donor transplants and most early series of right lobe grafts. Wachs et al. confirmed the feasibility of duct-to-duct biliary anastomosis and cite advantages such as minimization of bowel contamination, preservation of bilioenteric and bowel continuity, early return of bowel function, and elimination of the risk of internal herniation (22,48,49,50). Gondolesi et al. showed that there was no difference in the incidence of biliary complications between the two techniques (51). Liu et al. likewise demonstrated a similar incidence in biliary complications; however mortality appeared to be lower in the group with duct-to-duct reconstruction (0% vs. 11%) (48). Duct-to-duct biliary reconstruction appears to be gaining in popularity but it remains controversial as to whether there is any benefit over hepatico-jejunostomy.

Recipient Results

Recently published results of right lobe LDLT in adults are summarized in Table 57.2. The overall experience

with adult LDLT is still relatively small, but clearly patient and graft survival rates are better with right lobe grafts than left. Right lobe grafts appear to have comparable survival rates compared to whole cadaveric liver grafts as currently applied. A recent report from the NIH sponsored Adult-to-Adult Living donor Liver transplant (A2ALL)consortium, showed 1 year Kaplan Meier patient and graft survival was 89% and 81%, respectively in a cohort of 385 adult LDLT (52). Most series of living donor right lobe grafts report recipients of living donor grafts with lower MELD scores than cadaveric graft recipients. In the survey of Brown et al. 79% of LDLT recipients were not hospitalized at the time of transplantation and MELD scores were more frequently in the 11 to 20 range (41%) as compared to only 3.9% in the 21 to 30 range (37). The reason for limiting adult-to-adult LDLT to more elective patients is that recipients will have the maximum chance for survival. This was also the approach early in the experience of living donor transplantation for pediatric recipients. The severely ill recipient may not tolerate a smaller volume of hepatic mass than is provided with a whole organ cadaveric graft.

Table 57.2. Recent Results of Living Donor Right Lobe Liver T

Author	Center	Year	No. of recipient	Type of graft	Graft weight	Blood loss Donor (donor)	Pat Surv	Gr Surv	R cor
Miller	New York	2001	62	Right lobe	778 ± 224 gms		85%	77%	Bile
									Bili stri HAT
Marcos	Virginia	2000	40	Right lobe	790 ± 68 gms	699 ± 429 ml	90%	95%	Bili
									Sep
									Ble
Ghobrial	UCLA	2002	20	Right lobe			85%	95%	Ble
									Bile
									Bili stri 27%
Bak	Denver	2001	41	Right			88%		Bile

				lobe					30%
									Bili stri 12.
Olthoff	A2ALL	2005	385	Right lobe	966 ± 199 g		89%	81%	Infe
									Bile
									Bili stri
									Ree 25%
									HAT

Cohort divided into first 20 cases and next 20; figures are for the whole group.
HATS, hepatic artery thrombosis; UCLA, University of California, Los Angeles; A2ALL, Adult-to-A

Donor Complications after Liver Donation/Resection

While the mortality rate for living donors is less than 1%, morbidity is not uncommon. In the survey published by Brown et al. 14.5% of donors suffered one or more complications including biliary leak, the most common complication occurring in 6%, need for transfusion (5%), reoperation (4.5%), and major post operative infection (1%) (37). Following right hepatectomy the remnant native liver may demonstrate mild dysfunction including elevations of the transaminases, total bilirubin, and mild coagulopathy (34). The Kyoto group reported a mean peak total bilirubin of 3.2 mg/dL after right lobectomy that returned to normal within the first week (34). Others have reported coagulopathy with a mean peak prothrombin time of 18.7 seconds in the first 7 days after extended right lobectomy in living donors (29). Other surgical complications reported after right hepatic lobectomy include incisional hernia, biliary stricture, sepsis, pancreatitis and sepsis, and neuropraxia (22,33,34). In addition, pulmonary embolus has been reported after left lobe and left lateral segment living donation and clearly any patient undergoing right lobectomy would also be considered at risk for this complication (27,53).

Recipient Morbidity after Living Donor Right Lobe Transplantation

Biliary and vascular complications are the major morbidities associated with right lobe LDLT. The A2ALL study reported hepatic artery thrombosis (HAT) in 6% of recipients and portal vein thrombosis in 3% (52).A major vascular complication generally leads to loss of the graft. Biliary complications, including both leaks and strictures, occur in 15% to 60%, which is more frequent than after whole organ cadaveric liver transplantation (33,34). The A2ALL study reported bile leaks occurring in 32% and strictures in 17% of recipients (52). Other complications in recipients are similar to whole liver transplantation and include reoperation (25%), infections (41%), ascites (14%), hernia (6%), abscess (9%), bleeding (7%) and

pleural effusion (20%) (52).

Overview

Reduced-size liver grafts were an important step in the development of partial liver grafts. In large part, however, RSLT has been abandoned for techniques that increase the overall number of transplants that can be performed. Split-liver transplantation and adult LDLT using right lobe grafts are technically demanding procedures. Currently, only a few centers have gained significant experience with either technique. These methods of liver graft procurement differ from standard liver resection by the preservation of blood flow during hepatic parenchymal transection. Clearly when either split-liver transplantation or adult-to-adult living donor transplantation are performed by surgeons experienced with these techniques they can be performed safely with excellent graft function and limited donor morbidity or mortality. The introduction of these techniques has increased the number of available liver allografts for recipients; nonetheless there remains a large gap between the number of patients listed and the number of available donor livers. Perhaps in the future xenografts may fill this void.

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Chapter 58

Recurrent Disease Following Liver Transplantation

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Key Concepts

- Recurrent liver disease can occur for virtually all indications of liver transplantation.
- Hepatitis C virus (HCV) recurrence defined by histologic injury is almost universal and a significant proportion (20% to 30%) develops allograft cirrhosis by the fifth year post-transplant.
- HCV-related allograft cirrhosis is associated with a high rate of decompensation and mortality.
- The natural history of hepatitis B virus recurrence has been dramatically modified and outcome substantially improved by use of human immune globulin and nucleoside analogs.
- Recurrent autoimmune liver diseases (primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis) appear to have excellent intermediate patient and graft survival rates.
- The diagnosis of recurrent autoimmune liver diseases requires careful exclusion of alternative diagnoses (e.g., rejection, biliary obstruction) that can mimic the recurrent disease.
- Although recurrent alcohol use after transplantation for alcoholic liver disease is common, direct or indirect negative effects of recurrent alcohol use on the allograft is rarely seen.
- Recurrent Budd-Chiari syndrome can occur days to years after transplantation.

Liver transplantation is a well-established standard of care for many patients with end-stage chronic liver disease and acute liver failure. Despite excellent long-term survival, disease recurrence after liver transplantation is a relatively common problem. The clinical significance of this recurrence varies widely depending on the primary indication for liver transplantation. This chapter will primarily focus on disease recurrence after liver transplantation for viral hepatitis and autoimmune liver diseases with emphasis on natural history, risk factors for recurrence, diagnosis and management (Table 58.1). Significant attention will be paid to recurrent hepatitis C virus (HCV) infection, because currently HCV is the leading indication for transplantation and unlike other indications for liver transplantation, it is not a question of whether recurrence will happen, but how it will affect outcome and how best to manage or prevent recurrent viral hepatitis. Discussion on hepatitis B virus (HBV) will focus on the historical evolution of treatment with hepatitis B immune globulin, and more recently, the use of nucleoside analogs in preventing and managing disease recurrence. Clinically significant recurrent autoimmune liver diseases appear to be relatively rare; furthermore, study of this problem is limited by variable study design, small patient numbers, different immunosuppressive regimens, lack of long-term follow-up and established criteria and markers for diagnosing disease recurrence. Brief discussion will include recurrent alcoholic liver disease (ALD), a major contributor to the

development of end-stage liver disease in the United States, as well as to Budd-Chiari Syndrome (BCS). Recurrent hepatocellular carcinoma and cholangiocarcinoma are discussed in Chapters 44 and 45, respectively. In addition, the important issue of retransplantation for recurrent disease is discussed in detail in Chapter 59.

Table 58.1. Recurrent Diseases

Category	Incidence of recurrence	Diagnosis	Biopsy findings	Cholangiogram findings	
VIRAL HEPATITIS					
Hepatitis C	Viremia universal; 60%–80% acute hepatitis at 4–6 mo, chronic hepatitis 80%–100% at 1–4 y	HCV RNA by PCR	Periportal inflammation, lobular hepatitis	Normal	A r H ir h tl a h
Hepatitis B	Historically, without HBIG, 50%–85%; risk <10% with HBIG and lamivudine	HBsAg (often HBeAg-positive with high HBV DNA levels)	Periportal inflammation, lobular hepatitis	Normal	A r H ir h tl a h
AUTOIMMUNE COMPONENT					
Primary biliary cirrhosis	15%–25%	Biopsy and exclude alternative diagnoses	Florid duct lesion (granulomatous cholangitis) or destructive lymphocytic cholangitis within a dense portal infiltrate	Normal	A r G o ir
Primary sclerosing cholangitis	20%	Biopsy and/or cholangiography, exclude alternative diagnoses	Fibro-obliterative lesions and/or firbrous cholangitis with/without ductopenia, biliary fibrosis	Nonanastomotic extra/intrabiliary stricturing, beading and irregularity >90 d and diverticulum-like outpouchings	D r a s h tl n s

			or biliary cirrhosis		d i C p is
Autoimmune hepatitis	20%–30%	Biopsy, positive autoantibodies, and exclude alternative diagnoses	Periportal hepatitis, lobular inflammation	Normal	A r h (h tl b o

HCV, hepatitis C virus; RNA, ribonucleic acid; PCR, polymerase chain reaction; Peg-IFN, pegylat hepatitis B e antigen; DNA, deoxyribonucleic acid; GVHD, graft versus host disease; UDCA, urs Cytomegalovirus; HLA, human leukocyte antigen; LT, liver transplantation; AIH, autoimmune he

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Recurrent Viral Hepatitis

Hepatitis C Virus

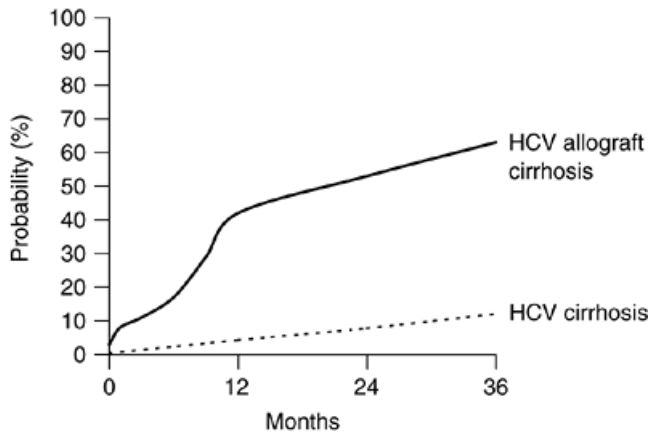
HCV-related end-stage liver disease is the most common indication for liver transplantation in the Western world. Recurrence of HCV infection after liver transplantation is nearly universal based on the presence of HCV ribonucleic acid (RNA) in the serum. During the anhepatic period HCV RNA levels typically decline to low and sometimes even undetectable levels (1,2). Four to 8 days after transplantation, viral levels often increase to levels 20-fold greater than in the pretransplant period (3), typically peaking between 1 and 3 months post-transplantation.

Evidence of histologic damage may occur in the non–HCV-infected recipient because of several causes including recurrent disease, however, abnormal liver biopsies are significantly higher in HCV-infected recipient than among controls (70% vs. 15% at 1 year post-transplantation; $P < 0.0001$) (4). Acute hepatitis occurs in 60% to 80% at a median of 4 to 6 months after transplantation and chronic hepatitis in 80% to 100% by 1 to 4 years (5,6,7). Acute recurrent HCV infection often causes increasing serum aminotransferases. Typically, liver biopsy is performed at this time to exclude acute cellular rejection (ACR), although uniform practice in this respect between transplant centers does not exist. Empiric treatment of ACR is not recommended as both ACR and recurrent HCV can improve with steroid boluses and steroid boluses have been shown to promote fibrosis progression in recurrent HCV (8,9). ACR often occurs within the changes of recurrent HCV; therefore often the distinction is not between recurrent HCV infection and ACR, but rather between ACR with or without underlying features of recurrent HCV infection. Table 58.2 outlines the histologic features of recurrent HCV and ACR (10,11). Following this acute HCV recurrence, chronic recurrent HCV develops, demonstrated by chronic hepatitis with variable degrees of mixed portal, periportal, and lobular inflammation with or without viable degrees of portal and/or periportal fibrosis. The severity of recurrent HCV should be defined by grade (necroinflammation) and stage (fibrosis).

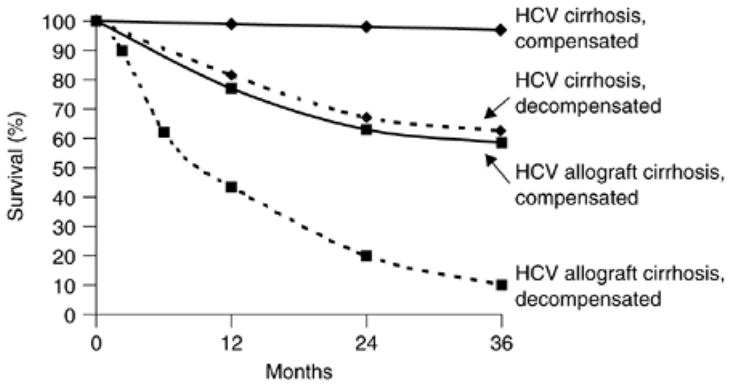
Table 58.2. Histologic Features of Recurrent Hepatitis C Virus Versus Acute

Cellular Rejection

	Recurrent hepatitis C virus (HCV)	Acute cellular rejection
Time post–liver transplantation	Anytime; usually within the first year	Usually in first 2 mo
Portal Inflammation	Most cases; mononuclear infiltrate	Always; mixed infiltrate
Aggregates	Usually	Occasionally
Follicles	50% of cases	Very rarely
Eosinophils	Inconspicuous	Almost always
Steatosis	Often	Never
Acidophilic bodies	Common	Uncommon
Duct damage	Approximately 50% of cases	Very common
Other	Bridging necrosis or fibrosis	Endotheliitis and central venulitis
Atypical features	Cholestasis, ballooning degeneration without significant inflammation, marked ductular proliferation mimicking obstruction, granuloma	Prominent periportal and lobular necroinflammatory activity without subendothelial venular inflammation



▪ **Figure 58.1** Probability of developing decompensated cirrhosis (ascites, encephalopathy, or variceal hemorrhage) from the time of diagnosis of hepatitis C virus (HCV) cirrhosis (pretransplant) and HCV allograft cirrhosis (post-transplant). (Adapted with permission from Berenguer M, Prieto M, Rayon JM, et al. Natural history of clinically compensated HCV-related graft cirrhosis following liver transplantation. *Hepatology* 2000;32:852–858; and Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472.)



▪ **Figure 58.2** Survival rates for compensated and decompensated hepatitis C virus (HCV) cirrhosis and HCV allograft cirrhosis. (Adapted with permission from Berenguer M, Prieto M, Rayon JM, et al. Natural history of clinically compensated HCV-related graft cirrhosis following liver transplantation. *Hepatology* 2000;32:852–858; and Fattovich G, Giustina G, Degos F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997;112:463–472.)

The natural history of chronic HCV is highly variable and poorly understood. For some, the natural history is accelerated compared with the nontransplant setting; 20% to 40% of patients transplanted for HCV develop allograft cirrhosis after only 5 years as compared to 3% to 20% after 20 years in the nontransplant setting (12,13,14,15). Once cirrhosis develops in the transplant setting, two-thirds will develop decompensation within 3 years (Fig. 58.1) (15,16). The development of decompensation is associated with a very poor outcome with

approximately only 10% surviving 3 years (Fig. 58.2) (15,16). Although nearly all patients develop some evidence of histologic recurrence, approximately a third will develop only minimal fibrosis after 5 years of follow-up (17). Although many studies have shown that short-term patient and graft survival is similar for patients undergoing liver transplantation for HCV compared to other indications, these studies were likely underpowered to detect small differences. Analysis of the United Network for Organ Sharing (UNOS) database revealed significantly diminished survival at 5 years after primary liver transplantation in HCV-positive patients (65.6% vs. 56.7% for HCV-negative transplant recipients; $P < 0.05$) (18).

The presence of rapidly progressive cholestatic HCV is observed in approximately 5% of patients transplanted for HCV, typically developing 1 to 3 months post–liver transplant and resulting in graft failure in 3 to 6 months (19). Characteristics of this syndrome have been outlined by a recent consensus conference (19). Patients typically have very high serum HCV RNA levels with serum bilirubin levels greater than 6g/dL and alkaline phosphatase

levels greater than five times the upper limit of normal. Patients should not have a history of hepatic artery thrombosis, nor any surgical biliary complications (i.e., normal cholangiogram). Liver biopsy often reveals ballooning of hepatocytes predominantly in the perivenular zone (not necrosis or fallout), paucity of inflammation, and variable degrees of cholangiolar proliferation without bile duct loss. The pathogenesis of this syndrome remains undefined, but preferential Th2 cytokine production by intrahepatic lymphocytes has been implicated (20,21). Optimum treatment remains uncertain, but is focused on reducing very high HCV RNA levels by reducing immunosuppression and indefinite use of interferon (22).

A number of factors have been identified that impact both severity of HCV recurrence as well as patient and graft survival. Table 58.3 outlines these viral, recipient, donor and post-transplant factors. The effect of HCV on living donor liver transplantation (LDLT) is controversial. Some studies have suggested earlier and more severe recurrent HCV, including cholestatic HCV, following LDLT compared to deceased donor controls (33,34,35), whereas others have suggested no difference in recurrence or patient or graft survival (36,37). Each

study has limitations, including small numbers, being from a single center, lack of protocol liver biopsies, and short-term follow-up. Results of the National Institutes of Health (NIH) sponsored multicenter Living Donor Liver Transplant Cohort Study will help clarify these issues. Another controversial issue in terms of HCV recurrence is the suggestion that the rate of fibrosis progression in recent years appears to be accelerating (38). There are many potential factors that could account for this finding including development of new immunosuppressive agents, reduction in overall immunosuppression, more frequent use of interferon therapy prior to liver transplantation, thereby selecting for more virulent HCV strains and use of older and marginal donors in patients with chronic HCV infection.

Table 58.3. Factors Associated with Severe Recurrence and Patient and Graft Survival After Liver Transplantation for Hepatitis C Virus

Viral	Recipient	Donor
Pre-LT viral load (23,24)	Age >60 y (25)	Age >50 y (26,27)
Early post-LT viral load (28)	Lack of $CD4^+$ response (29)	Warm ischemia time (30)
Pre-LT lack of IFN response (?)	Non-white (24,25)	Steatosis (?)
Post-LT	Female gender (18)	Cold ischemia time (?)
Treatment of rejection with steroids (8,9)	CMV infection (27,31)	HLA-matching (?)
Treatment of rejection with anti-lymphocyte preps (24,32)	Diabetes (25)	LDLT (?)
Rapid steroid taper (26)	Previous HCV Treatment (?)	HCV-positive (?)

LT, liver transplantation; ?, data either conflicting or unknown; CMV,

Cytomegalovirus; HLA, human leukocyte antigen; LDLT, living donor liver transplantation; HCV, hepatitis C virus.

The optimum immunosuppressive regimen for HCV-infected liver recipients remains to be defined. The use of high-dose maintenance steroids for prevention of ACR has been associated with decreased patient and graft survival compared to non–HCV-infected transplant patients (24). One consistent finding in the literature is the lack of significant differences in outcome in recipients treated with tacrolimus versus cyclosporine-based immunosuppression. The impact of mycophenolate mofetil on HCV recurrence is unclear and the effect of sirolimus has not been thoroughly studied.

Given the accelerated natural history of HCV recurrence, several approaches have been proposed to prevent or slow the progression to HCV-related graft failure. Current treatment strategies fall into three general categories: (i) Pretransplant antiviral therapy, (ii) preemptive therapy (prophylaxis) started in the early post-transplant period before the development of clinically apparent acute hepatitis, and (iii) post-transplant therapy at the time of diagnosis of acute hepatitis or for established and/or severe chronic hepatitis. With viral clearance post-transplant, both long-term absence of HCV RNA in the liver and marked histologic improvement (inflammatory scores much more so than fibrosis scores) have been described (39). Unfortunately, most published studies investigating the role of treating recurrent disease with interferon with or without ribavirin (no longer the standard of care) have been small, single center, uncontrolled trials with significant variability in patient selection and type and timing of antiviral therapy administered, and study endpoints evaluated (i.e., histologic response, end of treatment response [ETR], sustained virological response [SVR]). Rates of SVR are far less than those achieved in immunocompetent HCV-infected patients. Potential reasons for this difference include higher HCV RNA levels post–liver transplant, a high frequency of genotype 1 patients and the clinical status of liver transplant patients, especially in the early post-transplant period, leading to poor tolerability and need for frequent dose reductions. Approximately only 60% of transplant recipients are eligible for preemptive therapy with interferon and ribavirin and the need for dose reductions during therapy are frequent, occurring in 28% to 50% of treated patients (40,41). The major reason for dose reductions is cytopenia, seen both at baseline in many transplant patients and as a direct result of therapy. Use of growth factors to prevent this problem is not uncommon. Another problem in the transplant patient is renal insufficiency, limiting the use of ribavirin on account of its associated risk of hemolytic anemia. The risk of rejection with interferon therapy is controversial. Some studies have not noted an increased risk of rejection with interferon treatment (41,42,43,44), whereas others have described an increased risk (45,46). The exact prevalence and severity is therefore debatable. Whether rejection is more common with pegylated interferon remains to be proven.

Pretransplant antiviral therapy for HCV is discussed elsewhere. In terms of HCV-infected patients on the transplant waitlist, these patients are often deemed too ill to consider therapy. However, 93% of infected patients on the waitlist have Model for End-Stage Liver Diseases (MELD) scores of 18 or less (19,47). The arguments to consider therapy in cirrhotics pretransplantation is that clearance or suppression may eliminate risk of developing recurrent HCV and suppression of HCV viral load pretransplant might reduce disease severity post-transplant (48). Successfully treating patients on the waitlist with a

low, accelerating dosage regimen has been described (49). Of 124 patients with advanced HCV disease (mean MELD = 11.0 ± 3.7; Child-Pugh class B and C was 36% and 19%, respectively), 22% achieved a SVR (13%—genotype 1, 50%—nongenotype 1). Of the 47 patients in this cohort that underwent liver transplantation, 15 had undetectable HCV RNA prior to transplant and 12 remained HCV RNA negative at least 6 months post-transplant. Current consensus recommendations are to strongly consider treatment in patients with MELD scores less than 19 (Child-Turcotte-Pugh [CTP] <8) and in selected cases between MELD scores of 19 to 25 (CTP 8 to 11), especially in those with genotypes 2 and 3 (19).

The rationale for preemptive antiviral therapy is based on evidence from the nontransplant setting that low viral levels predict greater rates of SVR and high rates of clearance in treatment of acute hepatitis soon after an exposure (50). Preemptive therapy is strongly considered in patients undergoing retransplantation for rapidly progressive HCV disease and HCV-negative patients receiving a HCV-positive donor although data supporting these practices are limited. With the preemptive approach, antiviral therapy is typically started within 1 to 4 weeks of transplantation when viral levels are low and prior to histologic hepatitis. However, it is also during this time that patients are often more ill, at greatest risk for infection and rejection, and

more susceptible to the side effects of treatment. Data supporting this approach are scarce. Two randomized trials have evaluated the role of interferon in this setting (Table 58.4). In one study, histologic disease recurrence at 1 year was lower in the interferon-treated group than in the no-treatment group (8 of 30 vs. 22 of 41; $P = 0.01$) (42). In another study, the incidence of histologic recurrence did not differ, however, development of acute HCV hepatitis was delayed in the treatment group compared to the untreated group (408 days vs. 193 days; $P = 0.05$) (43). In a small uncontrolled trial of preemptive interferon and ribavirin started 3 weeks post–liver transplantation for 1 year, SVR was achieved in 33% (20% genotype 1, 100% genotype 2) (53). Investigation on the use of pegylated interferons in this setting did not show significant virological or histologic benefits (41). Poor tolerability and need for dose reductions in all these studies was high. Further well-designed randomized, controlled studies are needed to determine who is most likely to benefit from this treatment approach and whether the histologic improvements and reduction in HCV recurrence is beneficial in the long run. A recent consensus panel concluded that preemptive therapy remains to be defined and should only be used in unusual circumstances (19). Clearly, we need to identify early predictors of severe recurrence and develop a targeted approach that treats only a subset of patients.

Alternatively, many transplant centers follow protocols to initiate treatment when clinically significant evidence of recurrent HCV develops. Table 58.4 reviews the three randomized controlled treatment trials. With careful monitoring for graft fibrosis through use of protocol liver biopsies, antiviral treatment can be initiated at either the diagnosis of acute hepatitis C in the graft or at first sign of significant graft damage (e.g., stage 2 fibrosis). This latter approach balances the risk/benefit of treatment; avoiding the expense and toxic side effects of treatment with an overall SVR of approximately 20% (54) considering not all patients will develop severe recurrence. Delaying therapy theoretically allows most patients time to be in better physical shape and to be maintained on lower doses of immunosuppressants (less bone marrow suppression), thereby allowing full antiviral therapy. However, adverse events requiring dose modifications or cessation of therapy have been reported to occur in as many as two thirds of treated patients (55). Among the therapeutic approaches, combination therapy with pegylated interferon and ribavirin will probably yield the greatest potential for benefit in patients treated for established disease, assuming patients can tolerate therapy. Controlled data is currently lacking, but SVRs of 45% have been reported in a pilot study (56). Again, responders are often those with nongenotype 1 and patients who are able to complete therapy. Although, combination therapy may not achieve a SVR in most patients, the question remains whether fibrosis progression can be slowed with continued therapy, as suggested for nontransplant HCV. A small study using paired liver biopsies concluded that interferon therapy does not consistently improve allograft histology months to years after achieving viral clearance and does not improve liver histology at all in nonresponders (57). These results are consistent with our own experience, and we feel that unless virologic clearance is achieved, the natural history of HCV is not modified with antiviral therapy after

transplantation.

Unlike the effectiveness of hepatitis B immune globulin (HBIG) in preventing HBV recurrence after liver transplantation, treatment with hepatitis C immunoglobulin (HCIG) has not yet been shown to be effective in preventing graft reinfection. Its benefit was suggested by a study that showed the risk of HCV recurrence lower in HBV and HCV coinfected patients receiving HBIG prior to introduction of HCV screening (pre-1990) compared to after introduction of HCV screening (post-1990) (58). However, a randomized controlled trial for prevention of HCV recurrence found no benefit (59). Current data are inconclusive as to whether HCIG is effective in preventing HCV infection. Further study in this area is ongoing to determine if combination therapy to lower circulating HCV RNA levels might enhance the efficacy of HCIG.

For patients who either fail or do not tolerate antiviral therapy, the only option for those developing allograft failure from recurrent HCV is liver retransplantation (see Chapter 59), which has been associated with a number of complex issues. The current MELD allocation system favors medical urgency over a post-transplant outcome, without regard to whether a patient has already received an allograft. Maximizing utility (transplanting organs to those who will make the best use of them) in respect to retransplantation for recurrent HCV allograft failure may require placing more weight on post-transplant outcome, through the use of a more restrictive policy on retransplantation (60,61). While limiting access, a more selective approach will undoubtedly result in improved outcomes and better use of a scarce resource.

Hepatitis B Virus

HBV is a leading cause of morbidity and mortality worldwide, both from development of end-stage liver disease and as a risk factor for hepatocellular carcinoma. Currently HBV accounts for approximately 5% of all liver transplants performed in the United States, for both chronic and fulminant liver disease. Historically, liver transplantation for HBV had been associated with poor outcome due to HBV recurrence and graft

loss, leading to lack of support by some third-party payers. In the past 15 years there have been significant advances in the prevention and treatment of recurrent HBV after liver transplantation. Survival rates today for patients transplanted for HBV-related liver disease are now comparable and even exceeding other indications for liver transplantation (62).

Table 58.4. Randomized Controlled Trials Evaluating Efficacy of Antiviral Therapy for He Liver Transplantation

	Reference	No. of Patients (Tx/No Tx)	Treatment regimen	ETR (%)	SVR (%)	Histologic Response	Disc vs.
PREEMPTIVE TRIALS	Sheiner et al. 1998 (42)	35/46	IFN, 3 MU TIW × 12 mo	n/a[a]	n/a[a]	IFN group less likely to develop hepatitis, effect greatest with viral levels	26
	Singh et al. 1998 (43)	12/12	IFN, 3 MU TIW × 6 mo	n/a	0 vs. 0	No difference in hepatitis or	42(?

						outcome; time to diagnosis of hepatitis longer in IFN group	
	Reddy et al. 2002 (51)	21/11	IFN, 1.5 to 3 MU TIW + ribavirin 400—1,000 mg/d × 48 wk	n/a	16 vs. 0	No difference	31 (or decr
	Chalasani et al. 2005 (41)	26/28	PEG-IFN α-2a, 180 mcg × 48 wk	15 vs. 0	8 vs. 0	Change in fibrosis score at 48 wk, but not at 72 wk	31 v
TREATMENT TRIALS	Cotler et al. 2001 (52)	12/4	IFN, 3 MU daily × 12 mo	50% vs. 0	12 vs. 0	Inflammation improved in treated patients at the end of treatment	12 v
	Samuel et al. 2003 (44)	28/24	IFN, 3 MU TIW + ribavirin 800—1,200 mg/d × 48 wk	32 vs. 0	21 vs. 0	No significant histologic improvement	43 v
	Chalasani et al. 2005 (41)	33/32	PEG-IFN α-2a, 180 mcg × 48 wk	27 vs. 0	12 vs. 0	Lower increase in histologic activity index at 48 wk, but not at 72 wk	30 v

[a]Only HCV Ab(+) necessary to enter study. HCV RNA not known on all patients.
[b]All patients tolerated 1.5 MU daily.
ETR, end of treatment response; SVR, sustained virologic response; IFN, interferon; ?, informat
IFN, pegylated interferon.

For consistency in this chapter, the term *recurrent HBV* refers to HBsAg positivity post-

transplant in patients transplanted for HBV-related liver disease complications (cirrhosis, hepatocellular carcinoma, and fulminant failure). This definition implies de novo HBV infection in the allograft. This should be differentiated from *reactivation* of HBV, which refers to HBsAg positivity in patients transplanted for *non*-HBV related liver disease who receive a hepatitis B core antibody positive (anti-HBc (+)) donor liver.

The use of HBIG with or without use of a nucleoside analog (e.g., lamivudine and adefovir) has greatly improved the long-term graft survival following liver transplantation for HBV diagnoses. Prior to the use of HBIG, recurrence of HBV infection after transplantation was very high (>80% in patients alive for more than 2 months after liver transplantation was a major factor in 73% of all post–liver transplantation deaths beyond the first 60 days) (63). Once recurrence was established it often followed an aggressive course, progressing from severe chronic hepatitis to cirrhosis and graft failure within 1 to 2 years of reinfection and a 2-year mortality of 50% compared with 20% among patients transplanted for other liver diseases (64). A quarter of patients would develop an aggressive fibrosing cholestatic variant leading to rapid graft failure (64,65). Because of these poor outcomes with transplantation for HBV, many programs considered HBV infection (especially with viral replication) a relative contraindication to transplantation in the early 1990s.

A landmark multicenter European study of 334 liver transplant recipients with HBV helped identify three key determinants of HBV recurrence after liver transplantation: (i) Presence of HBV replication, (ii) type of HBV-related liver disease, and (iii) presence of passive immune prophylaxis using HBIG (66). Three-year actuarial risk of HBsAg recurrence among patients with HBV-related cirrhosis was 83% in patients seropositive for HBV deoxyribonucleic acid (DNA) at the time of transplant, 66% among those seronegative for HBV DNA and seropositive for HBeAg and 58% in those seronegative for both HBV DNA and HBeAg ($P < 0.05$). Three-year actuarial risk of recurrence was significantly higher among patients undergoing liver transplantation for HBV-related cirrhosis (67%) than among those undergoing transplantation for hepatitis delta virus–related cirrhosis (32%) or fulminant HBV (17%) ($P < 0.001$). Risk of recurrence was significantly reduced with long-term use of HBIG (>6 months) as compared to no immunoprophylaxis and short-term use (<6 months) ($P <0.001$). Multiple smaller studies have confirmed the European experience and have showed that the median rate of recurrent HBV infection in patients receiving long-term HBIG therapy was approximately 20% (range 5% to 50%) (67). Tables 58.5 and 58.6 summarize risk factors for HBV recurrence. As suggested by previous studies, Asians and non-Asians do not appear to have differences in rates of HBV recurrence (76).

Table 58.5. Risk Factors for Hepatitis B Virus Recurrence After Liver Transplantation

HBV DNA >100,000 copies/mL at time of LT (68)
Inadequate anti-HBs titers in patients with high viral load (69)
Development of immune escape mutants[a] (70, 71)
Lamivudine resistance pre-LT (72,73)
HBV precore mutants? (74,75)

HBV, hepatitis B virus; DNA, deoxyribonucleic acid; LT, liver transplantation; anti-HBs, hepatitis B surface antibody.
[a]Most common glycine to arginine substitution at codon 145 of the HBV S protein reducing binding to anti-HBs.

The mechanisms whereby HBIG protects the allograft against HBV recurrence are poorly understood. It has been hypothesized that HBIG protects naïve hepatocytes against HBV released from extrahepatic sites through blocking of a putative HBV receptor (77); alternatively, it may neutralize circulating virus through immune precipitation and immune complex formation (78). Use of HBIG does not lead to HBV eradication as HBV DNA by

polymerase chain reaction (PCR) can be detected in the liver, serum, and peripheral mononuclear cells of HBsAg-negative transplant recipients on HBIG (79,80).

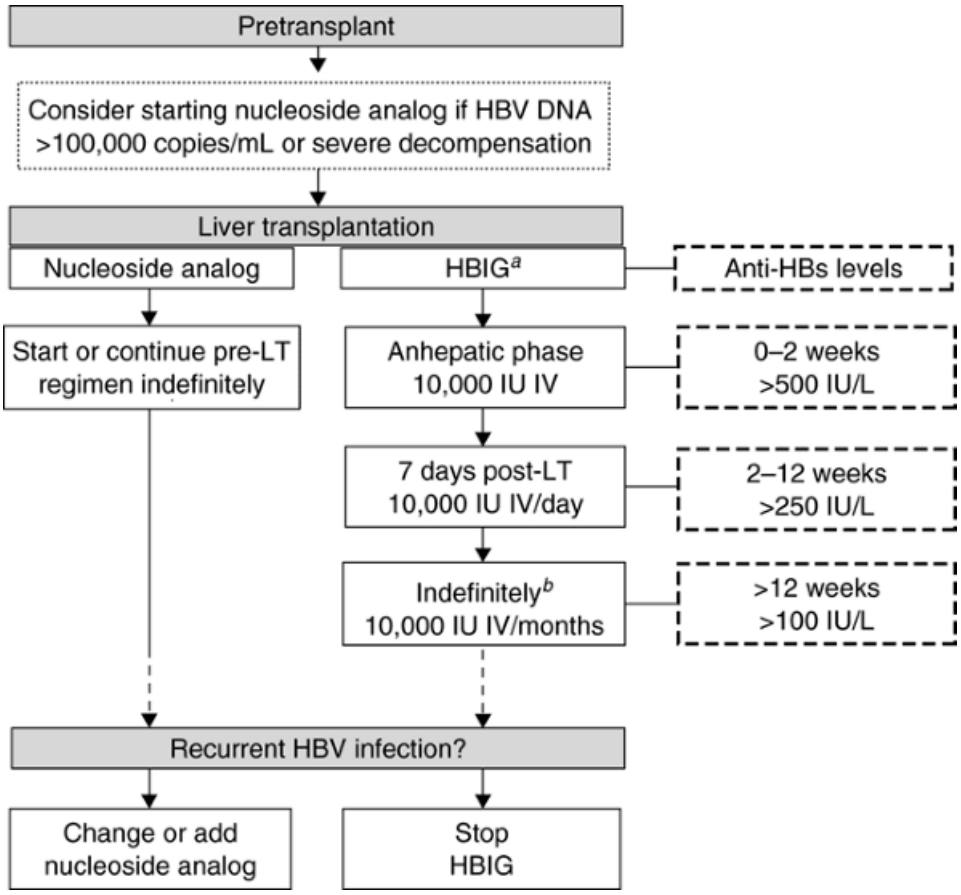
In the United States, most centers use a fixed dose regimen (Fig. 58.3). Unlike the regimen used by many European centers that maintains a trough hepatitis B surface antibody (anti-HBs) titer at 100 IU/L, the fixed dosing regimen results in a wide range of

anti-HBs titers with many trough titers greater than 500 IU/L (80). A high degree of interpatient variability in HBIG requirements is evident after transplantation. The anti-HBs levels outlined in Figure 58.3 (70) have been reported to reduce the recurrence rates of HBV infection to less than 20% (80). Patients who are HBeAg-positive pretransplant require more HBIG to achieve these targets, especially in the first week post-transplant. Unfortunately, the use of IV infusions of HBIG are very expensive (first year charges of >$100,000 and subsequent yearly charges of >$50,000) (81). Although previously associated with many potential side effects (immune-mediated reactions, anaphylaxis, mercury toxicity, and transmission of blood-borne infections), the new formulation of HBIG (NABI-HB; NABI, Rockville, MD) has largely eliminated safety and tolerability concerns. Although licensed for intramuscular use, if administered in large amounts, it can be used intravenously. Several centers use intramuscular HBIG rather than intravenous HBIG either from the beginning or transitioning to it months to years after transplant (82,83,84,85). The benefit of the intramuscular route is that the doses to achieve adequate titers are smaller; 4% to 15% of the 10,000 IU/month high-dose intravenous regimens (82,85). This dose reduction equates to significant cost savings; $52,600 per recurrence prevented compared to $371,000 per recurrence prevented for intramuscular HBIG plus lamivudine and intravenous plus lamivudine, respectively (85). A few differences between intramuscular and intravenous dosing include: (i) Intramuscular injection has a depot effect, so maximal intravenous levels are reached 3 to 5 days post-injection, (ii) 55% to 60% of the antibody diffuses into extravascular compartment, and (iii) the half-life of immunoglobulin G (IgG) given intramuscularly is 25 days, therefore monthly intervals can expect to maintain certain trough levels of anti-HBs (67). Alternative regimens to reduce HBIG requirements (and cost) have included lamivudine monotherapy, low-dose HBIG and lamivudine, and short-term HBIG plus long-term lamivudine, all at varying effectiveness.

Table 58.6. Rates of Hepatitis B Virus Recurrence Using Hepatitis B Immune Globulin in Terms of Initial Diagnosis for Liver Transplantation

Initial liver disease	Recurrence (%)
Acute hepatitis B	0–10
HBV-HDV coinfection cirrhosis	10–20
HBV cirrhosis without HBV replication	20–35
HBV cirrhosis with HBV replication	30–80

HBV, hepatitis B virus; HDV, hepatitis D virus.
From references: Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: a concise review. *Hepatology* 2000:32:1189–1195 and Samuel D, Muller R, Alexander G, et al. Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 1993;329:1842–1847.



▪ **Figure 58.3** Generalized summary of prophylaxis regimen to prevent hepatitis B virus (HBV) recurrence after liver transplantation. [a]Fixed dose regimen. [b]Alternatively some centers use IM hepatitis B immune globulin (HBIG) or stop IV HBIG after 6 to 24 months.

The development of nucleoside analogs has changed the current standard of care for preventing HBV recurrence. Lamivudine and adefovir are both safe and effective in liver transplant recipients. Both agents have been used alone and in combination with HBIG as prophylactic therapy (Fig. 58.3) and for patients who develop HBV recurrence after liver transplantation (86). The benefit of lamivudine is its low cost and excellent tolerability in cirrhotics and liver transplant recipients. The main disadvantage of lamivudine is resistance (20% after 1 year, 50% after 3 years) (87). Both wild-type and lamivudine-resistant HBV are sensitive to adefovir. Addition of adefovir to lamivudine can reduce HBV recurrence in patients transplanted with lamivudine resistance (86,88). Resistance to adefovir has been described, although the incidence of this resistance is low (3% in the first 2 years of therapy) (86). Entecavir was approved for treating chronic HBV infection in early 2005. Although clinical

studies in decompensated cirrhotics and liver transplant recipients have not been published, its safety profile and efficacy suggest it will be another antiviral agent to be used pre– and post–liver transplant. The incidence of entecavir resistance is low (1% after 1 year of treatment) and only in patients with preexisting lamivudine resistance (89). Tenofovir, approved for human immunodeficiency virus (HIV), has activity against wild-type and lamivudine-resistant HBV and appears to be safe and efficacious for use in decompensated cirrhosis and liver transplant recipients.

Given the associated increased risk of HBV recurrence with demonstrated viral replication pretransplant, it has become standard of care to start patients on nucleoside analogs prior

to transplantation and to continue their use post-transplant with the use of HBIG (combination therapy). Although use of antiviral therapy pretransplant has been shown to stabilize and improve liver function (in some cases obviating the need for transplantation all together), clinical improvement is slow and may not be helpful in very advanced disease (90,91,92). The optimum timing of initiating lamivudine pretransplant is unclear. Clinical effect can take 3 to 6 months, arguing for early initiation; however, risk for resistance increases with duration of therapy, negating the initial benefit and increasing risk of recurrent HBV after liver transplantation. The development of new nucleoside analogs effective against the YMDD mutation provides additional alternatives (93). The efficacy of lamivudine monotherapy to prevent recurrent HBV infection post-transplant is determined by the HBV DNA status of the patient at the time lamivudine is started. In the North American multicenter trial, 1- and 3-year post-transplant recurrence rates were 40% and 60%, respectively among patients who were HBV DNA positive before initiation of lamivudine therapy compared to 18% and approximately 20%, respectively, among patients who were HBV DNA negative before initiation of lamivudine therapy (94). Whether all patients in this later group of "nonreplicators" were truly HBV DNA undetectable is not known as a relatively insensitive assay (detection limit approximately 10^7 copies/mL) was used. In contrast, a Hong Kong study on lamivudine monotherapy reported only one of 26 patients (3.8%) developing reappearance of HBV DNA in serum after transplantation (58% were either HBV DNA or HBeAg-positive at time of transplant) (95). Some centers have shown good results with sequential prophylaxis with HBIG followed by lamivudine in patients who are HBeAg-negative and/or HBV DNA negative. In one study (96), 24 patients who received at least 6 months of HBIG were randomized to either HBIG (n = 12) or lamivudine (n = 12) for 1 year. Recurrent HBV occurred in 1 of 12 patients on HBIG therapy and 2 of 12 on lamivudine. Another study of 30 patients who were HBeAg-negative prior to transplant were considered for lamivudine substitution after 2 years of HBIG (97). Of the 16 patients who participated in the study, none developed evidence of HBV recurrence after a median follow-up of 13 months. Similar findings were seen in a prospective randomized trial comparing lamivudine monotherapy after 1 month of HBIG (n = 14) and lamivudine with long-term HBIG (n = 15) (98). After 18 months of follow-up all patients survived without HBV recurrence (all patients were HBV DNA negative at the time of transplant with or without use of lamivudine). Further studies are needed to define the subset of patients in whom HBIG can be discontinued and what the risk of lamivudine resistance and virologic breakthrough is with longer follow-up.

The combination of HBIG (either intravenously or intramuscularly) with a nucleoside analog appears to be more effective than either agent alone with rates of HBV recurrence ranging from 0% to 18% in patient populations with prevalence of HBeAg/HBV DNA positivity ranging from 34% to 90% (74,82,83,84,99,100,101). As a general rule, the studies showing the greater rates of recurrence (11% (101) and 18% (74)) used lower maintenance doses of HBIG and all patients who developed recurrence had lamivudine resistance prior to liver transplantation. Excluding these studies suggests that use of HBIG with lamivudine (or another nucleoside analog) offers the lowest rate of recurrence (<10%). The use of adefovir (95) (or entecavir) to treat lamivudine resistance pretransplant and post-transplant will likely enhance our current armamentarium to manage HBV in the transplant setting.

The effectiveness of passive immunoprophylaxis with use of HBIG at preventing HBV recurrence has led some to examine the role of active immunoprophylaxis (HBV vaccination) at preventing HBV recurrence post-transplant. There have been several reports on this issue, but their results have been conflicting. In two consecutive reports from Barcelona (102,103), 14 of 22 patients transplanted for HBV-related liver disease, who were receiving HBIG prophylaxis alone or in combination with lamivudine developed anti-HBs titers greater than 10 IU/mL after being immunized with one to two courses of double-dose recombinant HBV vaccine. Similarly, another Spanish group (104,105) reported of 9 of 12 patients developing anti-HBs titers greater than 10 IU/mL after HBV vaccination. In these studies, HBIG or HBIG with lamivudine was stopped and no HBV recurrence occurred over a mean postvaccination follow-up of 40 months. Another group reports similar success with 16 of 20 patients acquiring anti-HBs after vaccination allowing discontinuation of HBIG (106). Contrary to these successful reports, an Italian group describing their experience with HBV vaccination consisting of three, double doses of vaccine found that only 3 of 17 patients

developed postvaccination titers greater than 10 IU/mL (107). Possible reasons for these discrepant

results include the lack of patients with acute liver failure in the latter study, different vaccination scheduling, and the variable use of lamivudine. Nonetheless, it appears that successful vaccination followed by withdrawal of HBIG is possible, and further studies with larger number of patients and different forms of vaccine are needed to investigate these issues further.

Recurrence of HBV after transplant is defined by presence of HBsAg. Once recurrence develops, continued use of HBIG is not necessary as its role is purely a prophylactic one. The course of recurrent HBV has been modified by the availability of effective antiviral therapy. In a study of lamivudine in recurrent HBV infection, 60% had undetectable levels of HBV DNA after 52 weeks of therapy; however, 31% developed breakthrough infection secondary to lamivudine resistance (108). There are no current practice guidelines for treatment of recurrent HBV in transplant recipients, but given the need to avoid resistance in this population, the use of combination therapy is encouraged. If recurrence develops with use of HBIG alone, a nucleoside analog (lamivudine and/or adefovir) should be started. If recurrence develops in the setting of lamivudine use (i.e., presumed YMDD mutation), adefovir or entecavir should be added to lamivudine until viral suppression is achieved. Most investigators recommend a minimum of 3 months overlap (109). There is no data on the use of interferon in those nonresponsive or intolerant to nucleoside analogs.

Recurrent Autoimmune Liver Diseases

A number of diseases treated with liver transplantation are believed to be the result of immune dysregulation. Theoretically, disease recurrence of the autoimmune disease in the transplanted liver is prevented by the use of immunosuppressive therapy to prevent allograft rejection. For some, recurrence of the primary autoimmune disease can occur sometimes leading to allograft failure. This section will discuss the following recurrent diseases: Primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH).

Primary Biliary Cirrhosis

PBC is an immune-mediated disease leading to destruction and eventual loss of small intralobular bile ducts. This damage eventually leads to cholestasis and eventual cirrhosis and liver failure. Liver transplantation is a well-established treatment for PBC-induced end-stage liver disease with excellent long-term survival after liver transplant. Recurrent PBC after liver transplantation was first described in 1982 (110). Despite initial debate, it is now well accepted that PBC can recur after transplantation (111,112,113,114). Determination of recurrence rates has been difficult because most studies have not included protocol liver biopsies and disease recurrence is not always associated with cholestatic liver enzyme abnormalities. Moreover, the presence of cholestatic liver enzyme abnormalities may represent an alternative diagnosis after transplant (drug effect, infection or chronic rejection). Recurrence rates from the largest published series of 400 patients from Birmingham, England found histologic evidence of recurrence in 17% of patients at mean follow-up of 36 months (112), consistent with other series (115,116).

The gold standard for diagnosing recurrent PBC is liver biopsy. The key definitive diagnostic criterion is demonstration of a florid duct lesion (granulomatous cholangitis) or destructive lymphocytic cholangitis within a dense portal infiltrate (115). Sampling variations, particularly with early or mild recurrence may limit the identification of these findings. Less specific findings may be all that is identified, including mononuclear inflammatory infiltrate, formation of lymphoid aggregates, epithelioid granulomas, and bile duct loss. Recurrent PBC is considered definite if three of the four portal tract lesions are present in a patient transplanted for PBC with persistently positive antimitochondrial antibody (AMA) (113). It should be ensured that other diagnoses such as acute and chronic rejection, graft versus host disease (GVHD), biliary obstruction, viral hepatitis (Cytomegalovirus [CMV] and HCV), fungal infections and drug effects (sulfonamides, azathioprine, oral contraceptives, and penicillin-based antibiotics) are excluded. Although the AMA is the serologic hallmark of the

disease in the pretransplant setting, these antibodies can persist post-transplant, although there is typically a small and transient fall from pretransplant levels (117). Their presence does not correlate with clinical or histologic disease.

Risk factors for recurrence include younger donors, older recipients and prolonged warm ischemic time (112). The use of tacrolimus has also been implicated as a risk factor for PBC in one series (118), whereas azathioprine may have a protective effect (116). Whether this represents an adverse effect of tacrolimus or a benefit of azathioprine is not clear. Although graft failure from recurrent disease has been described (112,119), recurrence is not typically associated with graft loss. Although ursodeoxycholic acid (UDCA) is the mainstay of treatment of PBC pretransplant, its role in treatment of recurrent PBC has not been defined.

Primary Sclerosing Cholangitis

PSC is a chronic progressive disorder characterized by diffuse inflammation and fibrosis of medium and large intra- and extrahepatic bile ducts. This bile duct injury

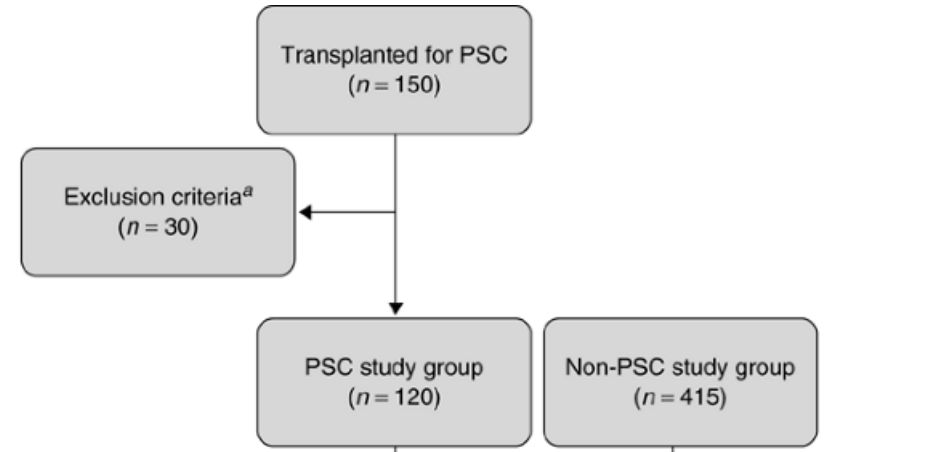
is most likely immunologic-mediated leading eventually to biliary obstruction and end-stage liver disease. Liver transplantation is the treatment of choice for patients with end-stage liver disease with 5-year survival rates as high as 85% (120,121). Diagnosis of PSC is made by demonstrating the characteristic biliary strictures on cholangiogram (diffuse, multifocal strictures and focal dilatation of the bile ducts, leading to a beaded appearance). A liver biopsy can confirm the diagnosis, but is rarely diagnostic due to a high incidence of sample variability. Liver biopsy findings include fibrous cholangitis (concentric rings of fibrous tissue with edema and inflammatory cells), bile duct obstruction, bile duct proliferation, and biliary cirrhosis).

The diagnosis of recurrent PSC is based largely on cholangiographic findings in combination with biochemical and histologic features of PSC. A large number of alternative diagnoses may lead to similar findings, and therefore, these diagnoses should be considered and excluded (Table 58.1).

Most patients with PSC undergo roux-en-Y loop anastomosis with removal of the recipient bile duct given the risk for cholangiocarcinoma in the native duct. These anastomoses are prone to biliary reflux and biliary infections, which can contribute to biliary stricturing. Studies comparing patients transplanted for PSC with those receiving an allograft with a roux-en-Y anastomosis have noted a greater frequency of nonanastomotic biliary strictures (122) and histologic features of biliary obstruction (123) in the PSC patients, supporting recurrence of PSC after liver transplantation.

Recurrence rates of PSC ranged from 8.6% to 20% with no associated reduction in patient or graft survival (120,124,125). The largest study of 120 patients transplanted for PSC compared outcome to 415 controls without PSC using strict cholangiographic and/or hepatic histologic criteria in which other causes of biliary strictures were excluded (Table 58.1 and Fig. 58.4) (125). In this Mayo Clinic study, of those with nonanastamotic lesions consistent with recurrent PSC, 59% had liver biopsies considered either marginal or not suspicious for PSC. Histologic evidence of recurrent PSC was noted in 11 (9.2%), 8 with fibrous cholangitis, and 3 with classic fibro-obliterative lesions at a mean of 1,380 days (range 420 to 3,240 days). In two patients without cholangiographic evidence of recurrence, both had advanced biliary-type fibrosis with ductopenia, which was otherwise unexplained and felt to be compatible with PSC. No specific risk factors for recurrence were identified. Patients fulfilling the criteria of recurrent PSC had significantly elevated serum alkaline phosphatase levels compared to the nonrecurrent group. In those with levels greater than 250 U/L after 1 year, recurrence should be suspected and cholangiogram and/or liver biopsy considered to

confirm diagnosis of recurrent PSC. No differences in patient or graft survival at 5 years in patients with recurrent PSC were noted, although patients with PSC recurrence may develop allograft failure (126,127,128).



Incidence of nonanastamotic strictures >90 days	18.3%	1.2%	$P < 0.001$
Histologic recurrence only	1.6%	n/a	
Days for nonanastamotic strictures to occur	360 ± 164 days	64 ± 89 days	$P < 0.001$
Graft survival at 5 years	79%	82%	P = NS
Patient survival at 5 years	86%	91%	P = NS

▪ **Figure 58.4** Summary of recurrent primary sclerosing cholangitis (PSC) after liver transplantation. [a]Excluded ductopenic rejection, anastomotic stricture alone, hepatic artery thrombosis, nonanastomatic strictures less than 90 days, ABO incompatibility, Cytomegalovirus (CMV) infection, prolonged ischemia. (Adapted from Sreekumar R, Gonzalez-Koch A, Moar-Kendler Y, et al. Early identification of recipients with progressive histologic recurrence of hepatitis C after liver transplantation. *Hepatology* 2000;32:1125–1130.)

There are no medical or surgical therapies to prevent or modify the rate of PSC recurrence. Whether differences to primary immunosuppression (cyclosporine vs. tacrolimus) regimens may modify or delay recurrence is not clear. A trend toward lower rates of recurrent PSC in early withdrawal of corticosteroids has been suggested (129). Whether corticosteroids avoidance only delays recurrence is not known. Use of OKT3 has been associated with higher risk of PSC recurrence (29% vs. 10%; odds ratio [OR] 3.7 [1.0 to 14.6]; P <0.05) (129). The use of high-dose UDCA (20 to 30 mg/kg) may have clinical benefits in the pretransplant setting (130), however, the potential benefits in patients transplanted for PSC is unknown.

Autoimmune Hepatitis

AIH represents a progressive, inflammatory disorder of the liver that may present as an acute hepatitis and liver failure. Patients with the diagnosis of AIH account for approximately 1.8% to 4% of liver transplant recipients (131,132). Compared to the general liver transplant population, these patients are more often young and female. Five-year patient survival after transplantation ranges from 82% to 92% (131,132). There does not appear to be a difference between patient and graft survival in patients with and without recurrent disease (131,132,133). Neuberger et al. reported the first case of post-transplant recurrence of AIH in 1984 when a 26-year old woman developed histologic and serologic evidence of AIH when corticosteroids were discontinued (134). The exact frequency of recurrence is unknown, and in early studies, was further confounded by lack of testing for HCV. Published reports have ranged from 2% to 42% (131,132,133,135,136,137,138) with 20% to 30% noted in most studies. A multicenter study of 72 cases of recurrent AIH

reported a median time to diagnosis of recurrence from 15 to 72 months (137). The recurrence rate appears to increase over time, possibly due to reduction in immunosuppression over time (8% at 1 year increasing to 67% at 5 years) (131).

Making the diagnosis of recurrent AIH is difficult given the lack of a single specific diagnostic marker. The diagnosis relies on a confident diagnosis of AIH as the primary indication for liver transplantation. Diagnostic criteria (139) for AIH have been established (see Chapter 31), and the diagnosis of recurrent AIH often involves using these same criteria. However, post-transplant, the use of immunosuppressive medications may influence the presence of biochemical and autoantibody markers. Recurrence can be indolent and only picked up by either routine laboratory testing or liver biopsy. Indeed, histologic abnormalities consistent with AIH on protocol liver biopsies have been detected when patients have no evidence of biochemical hepatitis (140,141). Although autoantibodies are often present, they are generally present at lower titers than they were pretransplant, possibly due to impaired autoantibody formation in the setting of immunosuppression. It is important to recognize that autoantibodies have also been seen de novo after liver transplant in the setting of rejection (142). Because diagnostic markers for recurrent AIH are lacking, the presence of characteristic histologic findings are important for making the diagnosis. While most often patients are clinically asymptomatic when recurrent AIH is diagnosed, the diagnosis is typically made when patients undergo liver biopsy in the setting of elevated liver enzymes to rule out rejection. For recurrent AIH, the biopsy should not reveal histologic features versus tacrolimus (endotheliitis or ductulitis), but rather a mononuclear infiltrate of lymphocytes and plasma cells extending from portal tract and into lobular parenchyma. With these findings it is important to exclude viral hepatitis (CMV, HBV, and HCV) and to consider exposure to hepatotoxic drugs. Histologic findings of recurrent AIH may be less well developed than AIH in the pretransplant setting because of immunosuppressive therapy. Differentiating recurrent AIH from rejection on biopsy, namely chronic rejection can be difficult, especially because chronic rejection may be a complication of recurrent AIH (143). There are also reports of late recurrence having been diagnosed by protocol liver biopsy in patients with normal liver enzymes (140). The diagnosis of recurrent AIH may depend more on exclusion of alternative diagnoses rather than identifying classical features of AIH.

Risk factors associated with the development of recurrent AIH include human leukocyte antigen (HLA) DR3-positive recipients (131,132,144), type 1 disease type (145), and suboptimal immunosuppression (131,134,136). Pretransplant necroinflammatory activity in patients with AIH, may also predict post-transplant disease recurrence (135). Prognosis in children may be worse than in adults (146).

Although there have been reports of successful steroid withdrawal in patients transplanted for AIH (147), numerous studies have described an association between recurrence of AIH with reduction in immunosuppression (134,136,148), suggesting that patients with AIH may require higher doses of immunosuppressive medication and prompting recommendations to wean steroids with caution. Further supporting the use of higher immunosuppressive dosing is the higher frequencies of ACR, steroid refractory rejection and chronic rejection in patients with AIH compared to patients with ALD (149) and genetic liver diseases (133). Whether cyclosporine versus

tacrolimus-based immunosuppressive regimens offer advantages is unknown. Lower incidences of ACR have been described in patients receiving a tacrolimus-based immunosuppressive regimen as compared to those receiving cyclosporine (149). Immunosuppressive management in AIH after liver transplantation requires further study.

Table 58.7. Predictors of Alcohol Relapse After Liver Transplantation

Abstinence <6 mo (150,151)	Absence of a life insurance policy[a](152)

Family history of alcoholism (153,154)	Prior alcohol rehabilitation (153)
History of drug abuse (152)	Daily alcohol consumption (155)
[a]Reflects stability of relationships and employment.	

Despite being more likely to have ACR during the early post-transplant period and reports of poor response to treatment (136), patients transplanted for AIH do not appear to have a significant difference in graft or patient survival (131,132,135,136), with exception of pediatric patients who have been reported to have rapid rates of recurrence frequently requiring retransplantation (146). Treatment of recurrence most often involves adjustments in immunosuppression. Recurrence of AIH does not appear to affect graft or patient survival (132), although longer follow-up is needed.

Recurrent Alcoholic Liver Disease

Alcohol is a major contributing factor in the development of end-stage liver disease with ALD representing the second most common indication for liver transplantation in the United States. Although ALD can be cured with transplantation, the disease of alcoholism remains and needs to be treated as such. Given the shortage of donor organs, alcohol use after transplant (recidivism) is a major concern to transplant centers. Many centers require that patients abstain from alcohol for at least 6 months before being listed for liver transplantation. This cutoff comes from studies suggesting abstinence of less than 6 months is a predictive factor for recidivism (150,151). Table 58.7 lists the factors predicting relapse of alcohol after liver transplantation.

Determining the incidence of alcohol use after liver transplantation is difficult given the highly variable means of detection (patient/family interviews, blood/urine testing, histologic findings) and different interpretations of recidivism (a single isolated drink to daily heavy use). A recent review of 30 published studies on alcohol use following liver transplantation for ALD using a definition of "any alcohol use" estimated a rate of approximately 20% (range 7% to 95%) at follow-up of 21 to 83 months following transplantation (156). This most likely is an under-representation.

Concerns that patients transplanted with ALD who resume moderate to heavy drinking will damage their transplanted livers either by developing recurrent ALD or by development of acute/chronic rejection because of noncompliance has not been supported by the literature (157,158,159,160,161), although a recent study has suggested that 10-year survival rates in patients with recidivism to be significantly lower than those without recidivism (45.1% vs. 85.5%, respectively; $P < 0.01$) (162). The high mortality rate in this study was explained by more frequent cancer and cardiovascular events. Although recidivism does not appear to directly lead to recurrent ALD, heavy alcohol use after transplantation can contribute significantly to other health problems. Patients transplanted for ALD are at a much greater risk of developing head and neck malignancies, especially if there is a history of heavy tobacco use. In addition, alcohol use in patients infected with HCV has potential to result in rapid recurrence of HCV in the allograft (163).

Recurrent Budd-Chiari Syndrome

Hepatic venous outflow obstruction at any level from the small hepatic veins to the junction of the inferior vena cava (IVC) and the right atrium is termed BCS (see Chapter 40). Most cases in western countries are the result of hypercoagulable states either related to an

underlying myloproliferative disease (MPD) or inherited thrombogenic diseases. Management is both medical (anticoagulation, diuretics, treating underlying hematologic disorder) and radiological/surgical (transjugular intrahepatic portosystemic shunt [TIPS] and liver transplantation). For patients undergoing transplantation, there is a risk of recurrence. The incidence of BCS after liver transplantation ranges from 0% to 27%, with a mean incidence of 8.5% (164,165,166,167,168,169). Often recurrence is associated with subtherapeutic anticoagulation. Recurrence typically develops months to years after transplantation, however, a recent report describes two cases of early recurrence (hours to days) despite initiation of anticoagulation within 24 hours of transplantation (169). Anticoagulation is not required for patients transplanted for protein C, protein S and antithrombin III deficiencies, as transplantation cures the underlying thrombogenic disorder. All other patients transplanted for BCS require long-term anticoagulation. A safe and effective alternative to anticoagulation in patients with underlying MPD is use of hydroxyurea and aspirin (168).

Summary

In summary, virtually all liver diseases that necessitate liver transplant can recur. Although recurrent disease has the potential to have the greatest impact on long-term outcomes after transplantation, the pace, frequency and severity of recurrence vary significantly according to the underlying disease. Precise predictors of who is likely to develop recurrence that threatens graft function remain largely undefined. For many with graft failure, retransplantation is the only option, and a number of groups have defined the parameters associated with outcome following retransplantation (see Chapter 59).

Annotated References

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Chapter 59
The Role of Retransplantation

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Key Concepts

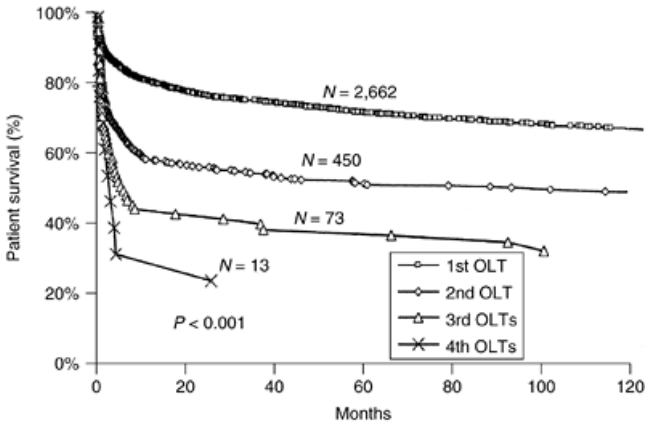
- Retransplantation after primary orthotopic liver transplantation (OLT) accounts for approximately 8% to 10% of all liver transplants in the United States.
- Overall graft and patient survival is lower following retransplantation compared with results after one transplant. The best outcome is achieved if retransplantation is undertaken immediately in the first week or at a much later stage after transplantation.
- Care should be taken in the decision to retransplant for recurrent disease, and should not be done in the setting of a severely decompensated patient who has poor functional status, high Model for End-Stage Liver Disease (MELD) scores, is advanced in age, on ventilator dependence, or on dialysis.
- Transplantation for recurrent hepatitis C remains controversial, however the presence of hepatitis C virus (HCV) in and of itself should not be a contraindication to retransplantation.
- Models to predict the outcome after retransplantation, which take into account patient status, disease severity, and donor graft characteristics should be further developed and validated.
- Although retransplantation offers outcomes that are inferior to primary transplantation, it should still be offered as potential life-saving therapy in cases of acute graft dysfunction, or in well-selected cases of recurrent disease and chronic graft failure.

The great success of liver transplantation since the 1980s has led to a rapid increase in the number of patients on the waiting list, with an unmatched increase in the number of cadaveric or living donors available. This gap continues to widen with the most recent published statistics showing over 17,000 on the waitlist in 2003, with only 5,364 transplants performed (1). Because of this disparity, the process of prioritizing individual patients for organ allocation is a constant source of debate, and critical in the discussion of appropriate allocation of livers to patients with a failed first graft. Despite increasing improvements in medical decision making, surgical technique, intensive care, and immunosuppression, a certain percentage of patients still experience acute or chronic graft failure and therefore require retransplantation—accounting for approximately 8% to 10% of all transplants performed in the United States per year. In 2003, 478 recipients (8.9%) had a prior liver transplant. During this same period, 1,819 patients died while still awaiting a first liver (the Scientific Registry of Transplant Recipients [SRTR] 2004 Annual Report). In addition, the outcome after retransplantation is not as successful as with primary transplantation in general, with long-term survival decreasing with each successive transplant (Fig. 59.1) (2).

Liver retransplantation not only poses a clinical and technical challenge, but also brings to bear serious financial and ethical issues because of increased costs and a finite number of available donors. Hospital charges are significantly higher and the length of stay is longer

for patients receiving a second transplant and

there is an obligatory net loss from the donor organ pool for patients who may have a greater chance of survival (3,4). In this chapter we will address the reasons for retransplantation, technical considerations, factors that contribute to the outcome after retransplantation, and also briefly explore the ethics of retransplantation.



▪ **Figure 59.1** Survival estimates of recipients undergoing multiple liver transplantations. Recipient survival after primary orthotopic liver transplantation (OLT) was compared with survival after transplantation with second, third, or fourth OLTs in a single-center series from the University of California at Los Angeles (UCLA) spanning two decades (2). (Reprinted from Busuttil RW, Farmer DG, Yersiz H, et al. Analysis of long-term outcomes of 3,200 liver transplantations over two decades: a single-center experience. *Ann Surg* 2005; 241(6):905–916.From Lippincott Williams & Wilkins.)

Rate of Retransplantation and Indications

The overall reported rate of retransplantation in the past 10 years in individual centers seems to vary between 7% and 17% (2,4,5,6,7,8,9,10,11,12,13). These rates have not remained constant over the years. The University of Pittsburgh studied the rates and causes of retransplantation in three eras—early eighties, late eighties, and nineties. The overall rate of retransplantation declined significantly over time: From 33% in the early eighties to 13% in the nineties (10), likely the result of improved clinical judgment, immunosuppression, advanced technical skills, and better antiviral medications. The overall rate of retransplantation in the United States, in a cohort of patients who underwent transplantation, in 2001 and 2002 was 9.4% of all patients receiving a deceased donor liver transplant (the US Organ Procurement and Transplantation Network [OPTN] and SRTR 2004 Annual Report).

Just as the overall rate of retransplantation has changed over time, so have the indications for transplantation. There has been a marked decrease in the rate of retransplantation for acute and chronic rejection. According to a series of 114 retransplants performed in Germany, the major causes of retransplantation during the early 1980s were acute and chronic rejection, with an incidence of 27% in each (14). However, in a more recent series, UCLA identified primary nonfunction (PNF) as the primary cause, accounting for over 25% of all retransplantation cases (15). According to data from a retrospective study of retransplantation at Pittsburgh over a 19-year period, the rate of retransplantation for rejection declined from 13.2% to 1%, and the rate of retransplantation for hepatic artery thrombosis (HAT) declined from 8.1% to 3.8%, with the rate for PNF increasing from 4.6% to 6.0% (10).

Retransplantation is also a significant event after pediatric liver transplantation, usually for different indications than adults, often for technical reasons. The incidence of

retransplantation in children is higher than in adults, with a range of 15% to 29% (16,17,18). The use of split and reduced size grafts, the small size of donors and recipients, and noncompliance in the teen years, are predisposing factors for an increased retransplantation rate in this population. The willingness of transplant surgeons and hepatologists to give more than one graft to children, or even more than two grafts, is greater, and fortunately, outcome is significantly better in the pediatric population as well.

The indications for retransplantation for adults and children can essentially be divided into two groups—those patients needing emergent retransplantation in the early days and weeks following transplant due to graft failure secondary to graft dysfunction or technical complications, and those requiring late retransplantation due to chronic rejection, recurrence

of disease, or late technical complications from vascular or biliary issues.

Indications for Early Retransplantation

Primary Graft Dysfunction

Early graft dysfunction is a term that is used to describe a spectrum of clinical conditions in which the transplanted allograft fails to provide adequate metabolic and/or synthetic function. These patients present with various degrees of hemodynamic and metabolic instability, and the potential for the development of multiorgan system failure. The severity of global hepatic dysfunction may be assessed by biochemical and metabolic markers including elevated transaminases, persistence of metabolic acidosis, elevated international normalized ratio (INR), and rising bilirubin. A diagnosis of PNF is a diagnosis of exclusion made if a graft shows complete lack of initial function and its dysfunction cannot be attributed to technical or other recipient causes, and is the most common cause of retransplantation in the initial days after OLT. A more stringent definition is used for the allocation of a second organ, where PNF is defined as graft dysfunction within the first week post-transplant leading to the death of the patient or retransplantation. Requirements include aspartate aminotransferase (AST) of value 5,000 or more, and either INR of value 2.5 or more, pH less than 7.3, or lactate value 4.0 or more. This allows for rapid relisting of patients at a Status 1 designation that places the patient at the top of the allocation list. Other grafts may present with a less dramatic picture, demonstrating prolonged cholestasis, evidence of ischemic/harvest injury on biopsy, and slow or inadequate restoration of function. Termed *early allograft dysfunction* (EAD) or delayed nonfunction (DNF) these grafts may require retransplantation if they fail to recover, although they often do not meet Status 1 criteria. These patients often have prolonged intensive care unit (ICU) stays with an increased risk of serious infectious complications (19,20).

The use of “marginal,” or extended criteria donor (ECD) grafts, that is, from higher risk donors based on demographic, clinical, laboratory, or histologic data, may increase the risk for early graft dysfunction. Although the definition of ECD is not clearly defined, grafts that may have an increased risk of graft dysfunction usually include livers from older donors, death-after-cardiac-death (DCD) donors, steatotic livers, split liver grafts, and those with prolonged cold ischemic times. Each of these factors has been associated with decreased graft survival, thereby leading to an increased potential need for retransplantation. There has been a steady increase in the use of ECD organs as the gap grows between recipients and the number of livers available. For example, there were 519 donor liver grafts over 65 (9.1% of all liver grafts) in 2003, compared to only 164 older grafts (4% of all grafts) in 1994, whereas the number of DCD donors increased by 43% between 2002 and 2003 (21). An increased rate of PNF has been associated with an increase in the utilization of donor organs from donors above 50 years of age from 1.5% in the early eighties to 22.5% in the nineties (10). From an ethical perspective, it is precisely the availability of retransplantation at a high status that allows for the use of these donors by providing a safety net if the ECD liver does not function properly. Previously, these grafts were used in patients in desperate situations and were associated with dismal graft and patient survival. As experience has been gained and results analyzed, it is now generally accepted that these grafts should be used in less critically ill recipients in order to obtain acceptable outcomes, as more stable patients may be able to better tolerate a period of relative graft dysfunction, or the need for

a retransplant procedure (22). Although this matching of donor to recipient is ideal, it is often not possible with our current allocation system that does not factor in graft quality.

Hepatic Artery Thrombosis

Acute HAT, despite a decreasing incidence (approximately 5%), is still a devastating problem that often requires retransplantation and is associated with markedly increased morbidity and mortality. In a review of 15 European liver transplant centers, the incidence of HAT was less than 5% but mortality rate was as high as 55% and the retransplantation rate was approximately 80% (23). The use of fibrinolysis, surgical thrombectomy, and immediate revascularization may avoid the necessity of retransplantation in some instances, but most patients still need a second graft. Previously patients with HAT were given Status 1 if diagnosed in the first week. However, recent data has shown that patients with HAT have a significantly lower risk of death while awaiting retransplantation than those requiring transplantation for other Status 1 designations (fulminant failure or PNF) (24), therefore, HAT can only be given Status 1A in adults if associated with a fulminant type of hepatic failure in the first 7 days (up to 14 days in children). Beyond 7 days, increased Model for End-Stage Liver Disease (MELD) exception points up to 40 may be requested.

Indications for Late Retransplantation

Chronic Rejection

Current modalities in immunosuppression have essentially eliminated the need for retransplantation for acute

rejection, and the incidence of retransplantation for chronic rejection has declined significantly. However, it still remains a significant cause in the late retransplant group. In a recent large series from Gainesville, Florida, 27% of late retransplants in both children and adults were for chronic rejection (25), and the Kyoto group reported a 35% rate of chronic rejection in a series of living donor liver transplants as a cause for primary graft loss requiring retransplantation (26).

Primary Biliary Cirrhosis

Although there was initially some controversy, recurrence of primary biliary cirrhosis (PBC) has been reported and is estimated at about 10% to 20%, occurring on an average 3 to 6 years after transplantation. Progression of recurrent PBC is often slow and may not necessitate retransplantation (27). In a series from the University of California, San Francisco (UCSF), recurrence of PBC was seen in eight patients with three graft failures that resulted in one death, one relisting for transplant, and one successful retransplant (28).

Primary Sclerosing Cholangitis

Histologic evidence of recurrence of primary sclerosing cholangitis (PSC) has been documented with rates ranging from 14% to 41% post-OLT (29). Injury to the bile ducts due to anastomotic, ischemic, and infection-related strictures may present similarly to PSC, therefore these causes of bile duct damage must first be ruled out. In a study of the United Network of Organ Sharing (UNOS) database of 2,154 patients with PSC, 315 (14.6%) required retransplantation (30). It was postulated that the reasons behind this were not just disease recurrence, but also a high rate of biliary complications and chronic rejection.

Autoimmune Hepatitis

Liver transplantation for autoimmune hepatitis (AIH) is highly successful; however, there is a high risk of rejection and severe recurrent AIH. Recurrence rates range from 16% to 46%. Treatment with steroids and increased immunosuppression may control disease progression, but a significant number of patients may require retransplantation (31).

Nonalcoholic Steatohepatitis

Small case series have demonstrated recurrent nonalcoholic steatohepatitis (NASH) in 10%

to 47% of patients post-OLT (32,33). Diabetes mellitus, hyperlipidemia, and obesity, often associated with NASH, are common after transplantation and progression from fatty liver to cirrhosis post-OLT has been documented in serial biopsies, with cumulative steroid use being a potential risk factor (33). There is very little, if any, data on retransplantation for recurrent NASH, but as the percentage of patients that are transplanted for this disease increases in the United States, it may become a very significant concern.

Alcoholic Liver Disease

Alcoholic liver disease is the second most common cause of liver failure leading to OLT. Multiple studies have looked at recidivism rates that may be as high as 30% to 40%, but the recurrence of alcohol-induced cirrhosis and liver failure is rare (34). Earlier arguments that suggested that alcohol recidivism would lead to poor adherence to immunosuppressive regimens and premature graft loss, have also been proven false (35). Obviously, resumption of alcohol use would be a contraindication for re-OLT and careful assessment of the psychosocial situation is necessary before approval in this patient group.

Hepatitis B Virus

Historically, recurrent hepatitis B virus (HBV) after OLT resulted in rapidly progressive hepatic deterioration and extremely high mortality rates (36). Prophylaxis with hepatitis B immunoglobulin (HBIg) and oral nucleoside therapy has greatly decreased the recurrence rate and made survival following OLT for Hepatitis B comparable to that of other causes of liver disease (37). However, occasional recurrence evolves (approximately 5%), mainly due to acquired HBV mutations (38), but retransplantation for recurrent disease is now rare.

Hepatitis C Virus

Cirrhosis from hepatitis C virus (HCV) is now the most common indication for liver transplantation in the United States (39,40). After liver transplantation, HCV can be detected in the serum of nearly all patients who had HCV before transplant, although the degree of histologic recurrence is highly variable (41). Hepatitis C viral RNA levels may increase up to ten times of pre-OLT levels at 4 weeks after transplantation and hepatic fibrosis may have an accelerated course (42,43,44,45). Unlike HBV, no therapy has been conclusively shown to alter HCV recurrence or disease progression, although some progress has been made in antiviral therapy (46,47). The natural history of post-transplant HCV infection is quite indolent but approximately 20% may have continuing graft damage progressing to cirrhosis in 5 to 10 years, and this percentage may be increasing (48,49).

Results of Retransplantation

Comparison to Primary Transplantation

Early studies in liver transplantation showed significantly worse patient and graft survival after retransplantation when compared to primary transplants. Along with the improvement in the results of primary grafting, a comparable trend is noted with the outcome of retransplantation. However, when all patients are included, the results with retransplantation still fall short of primary grafting (2,50,51). Recent data from the SRTR show that patients who underwent transplantation between 2001 and 2002 who had prior transplants have lower survival than those receiving an initial transplant (OPTN/SRTR 2004 Annual Report—Table 59.1).

Timing of Retransplantation

Several analyses have shown that timing plays an important role in the outcome of retransplantation (5,50,52,53). The series from UCLA demonstrated that the survival of patients who underwent transplantation within 1 week is nearly equivalent to that seen in patients who underwent retransplantation at a much later date. It was the patients who underwent retransplantation in the period between 8 and 30 days who had a significantly worse outcome, emphasizing the need for early recognition of patients who require early

retransplantation.

There is a clear differential outcome between those requiring early urgent retransplantation and those who require an “elective” second graft. In some cases, the patients who underwent retransplantation many months after the primary transplant exhibit survival curves similar to those receiving a single transplant. For urgent retransplantation, which likely coincides with the first 30 days after surgery and is likely due to primary graft dysfunction, hospital charges are higher, the length of stay longer, and survival worse (4). Although easier from a technical point of view, the mortality is likely high because of the poor clinical condition of the recipient (9).

Outcomes of Retransplantation in Hepatitis C Virus Patients

This remains a controversial subject with a great difference of opinion between centers. Early reports suggested that retransplant in HCV patients had a significantly worse outcome compared with other diseases with the presence of HCV being an independent risk factor for increased mortality (44,54,55,56,57). The presence of early fibrosing cholestatic hepatitis was considered a contraindication for retransplantation by many centers. Looking carefully at some of the earlier reports, it is found that many of the postoperative deaths were in the first 90 days, perhaps reflecting not just death from severe recurrent HCV, but also the poor perioperative condition of the patient with recurrent HCV undergoing retransplantation. Some centers have supported retransplantation for recurrent HCV if retransplantation was performed early in the course of recurrent disease (54). Others have reported that it is the physical condition of the HCV-infected patient at the time of retransplantation that is most predictive of outcome (58). A recent report from the SRTR database assessed the relative effect of HCV diagnosis on mortality after retransplantation demonstrating that retransplant recipients with HCV had a 30% higher covariate-adjusted mortality risk than those without HCV (59) (Fig. 59.2). Of concern was the fact that the increased risk associated with mortality was concentrated in younger patients (age 18 to 39) and in patients transplanted in more recent years (2000 to 2002), perhaps reflecting a willingness of transplant centers to perform retransplantation in younger patients who may be more severely ill. This study, however, did not compare outcomes between recipients with HCV and other specific disorders, and inherent differences between cohorts may have less importance than the degree of illness at the time of re-OLT (60).

Although retransplantation for HCV-positive patients accounts for approximately 40% of all retransplants in the US, and is predicted to increase in number, most second transplants are performed for causes other than recurrent disease. In some reports there appears to be little difference in the survival rate for HCV patients undergoing re-OLT when compared with other causes of re-OLT, if all other variables are kept equal (61,62), and therefore retransplantation should be considered a potential option for the treatment of recurrent disease. However, these patients require a more careful decision-making process when compared to non-HCV patients, and re-OLT is best performed before severe decompensation (51,63). This can be a difficult task in the current system of liver allocation that provides grafts to the “sickest first.”

Cause of Death Following Retransplantation

The development of sepsis and multiorgan failure accounts for most of the deaths in the patients undergoing retransplantation, and the largest proportion of deaths occur in the first few weeks after transplant. The

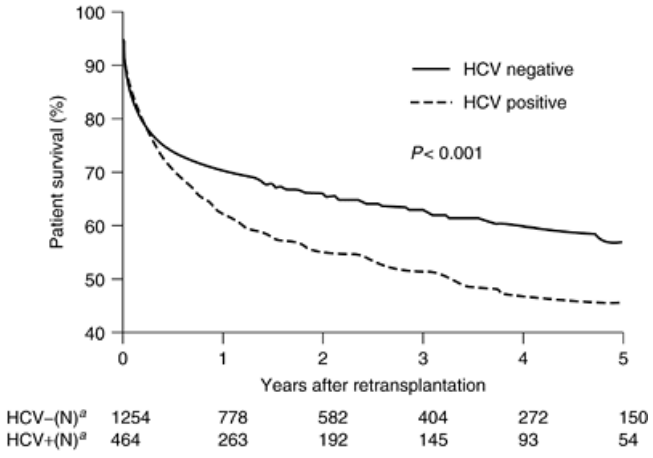
incidence of death secondary to sepsis is twice as high in patients undergoing retransplantation compared with those receiving just one graft with a 50% incidence of fungal infection (55,64). In a recent series from UCSF, 28% of patients who underwent retransplantation experienced serious infections in the first month, and 17.5% died within the first 6 months from multiorgan dysfunction associated with sepsis or poor graft function (65). Roayaie et al. reported that approximately two thirds of deaths after retransplant for HCV recurrence were associated with infection (57). The high incidence of graft loss due to

sepsis in these patients may reflect the immunosuppressed status of the patients prior to retransplantation, as well as their deteriorated functional status. In light of these findings, it may be prudent to reduce immunosuppression perioperatively, and initiate more effective antimicrobial prophylaxis for patients undergoing retransplantation. Less frequent causes of death following retransplantation include technical problems such as intraoperative mortality, arterial and portal vein thrombosis, and postoperative complications such as cardiac events, neurologic complications, recurrent disease, and persistent liver failure.

Table 59.1. Unadjusted Graft Survival, Deceased Donor Liver Transplants, Primary Ver Survival at 3 Months, 1 Year, 3 Years, and 5 Years

		3 months			1 year			3 years		
		N	%	Std. (%)	*N*	%	Std. (%)	*N*	%	Std. (%)
Total	**All**	9,131	88.2	0.3	9,131	81.4	0.4	8,697	72.2	0.5
Previous	**No**	8,275	89.4	0.3	8,275	83.1	0.4	7,863	74.1	0.5
Liver Tx	**Yes**	856	77.0	1.4	856	65.4	1.6	834	54.7	1.7

OPTN/SRTR 2004 Annual Report, Table 9.9a—Data as of May 3, 2004. Cohorts are transplants p 2002 for 3 month and 1 year; 1999–2000 for 3-years; and 1997–1998 for 5-year survival. Graf individual transplants until graft failure.
The data and analyses reported in the 2004 Annual Report of the U.S. Organ Procurement and T Network and the Scientific Registry of Transplant Recipients have been supplied by UNOS and U with HHS. The authors alone are responsible for reporting and interpreting these data.
2004 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scient Transplant Recipients: Transplant Data 1994–2003. Department of Health and Human Services, Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD Organ Sharing, Richmond, VA; University Renal Research and Education Association, Ann Arbor,



▪ **Figure 59.2** Kaplan-Meier curve comparing unadjusted mortality between hepatitis C virus (HCV)-positive and HCV-negative liver retransplant recipients in a cohort of patients who underwent retransplantation between 1997 and 2002 using the Scientific

Registry of Transplant Recipient (SRTR) database (59). [a]Denotes number of patients at risk at each corresponding time. (Reprinted from Pelletier SJ, Schaubel DE, Punch JD, et al. Hepatitis C is a risk factor for death after liver retransplantation. *Liver Transpl* 2005;11(44):434–440.American Association for the Study of Liver Diseases.)

Predictors of Mortality After Retransplantation

In an attempt to maximize the use of valuable organs, there have been many efforts to determine which factors are associated with the outcome in order to develop a model that might accurately predict survival in patients undergoing liver retransplantation. It has become increasingly apparent that it is not just the quality of the donor, but the status of the recipient that contributes most to the outcome after retransplantation. A multivariate analysis performed at UCLA determined that donor cold ischemia time over 12 hours, preoperative mechanical ventilator requirement, preoperative serum creatinine greater than 1.6, and serum total bilirubin over 13 were all independent risk factors predictive of a patient's poor outcome (53,64). Rosen and Martin identified bilirubin and creatinine as predictive of poor outcome in HCV patients (56). The University of Pittsburgh identified older donor age, gender, and choice of immunosuppression as predictive of poor outcome (52). In a series from Mount Sinai Medical Center, recipient age over 50, a preoperative creatinine greater than 2, and the use of intraoperative blood products, significantly impacted the survival of those patients requiring retransplantation over 6 months after the primary transplant (55).

Models to Predict Survival After Retransplantation

The critical shortage of donor organs and the resultant prolonged patient waiting periods before transplantation have prompted many to define a mathematic model that adequately predicts survival after retransplantation. Rosen et al. assigned a mortality risk score based on preoperative variables in patients undergoing retransplantation, which included recipient age, creatinine, bilirubin, non-PNF diagnosis, and UNOS

status (66). Individual patients were then stratified into low-, medium-, and high-risk groups, in an attempt to predict survival, finding recipient age, creatinine and bilirubin levels, and timing of transplant between 15 and 60 days as being predictive of outcome. These findings were validated in a multicenter international study (67). Another model put forth by Markmann et al. from the UCLA database, similarly employed a scoring system utilizing five noninvasive and readily available clinical parameters, including recipient age, creatinine, bilirubin, cold ischemia time, and ventilatory status (64). Patients having four out of a possible five points had a 1-year survival of only 27%. This classification system adequately discriminated high-risk and low-survival patients in three databases to which it was applied (UCLA, Baylor University Medical Center, and the UNOS registry). More recently, a universal model for predicting survival was proposed by Ghobrial et al. for both primary transplants and retransplants hoping to provide guidance for tailoring specific organ needs to specific recipients on the basis of the severity of disease and expected outcomes, which incorporated MELD score, recipient age, and timing of transplantation (63). Interestingly, none of these models found the diagnosis of HCV as a poor prognostic indicator in multivariate analysis. Applications of models such as these can theoretically result in better decision making, improved survival, and an increased efficacy in organ utilization.

Meld Score and Retransplantation

In primary transplantation, MELD score has not correlated with post-transplant outcome except at the very highest of MELD scores (68). However, in the population who undergo retransplantation, MELD score may have a more significant impact, although there is some disagreement here as well (69). MELD scores correlated with survival outcome in patients who underwent retransplantation at the University of Nebraska (70). In the multicenter

study by Rosen, the MELD score was predictive of survival in patients undergoing retransplantation after 15 days (67). In a recent paper by Yao et al. there was a trend for a correlation between CTP and MELD scores with 1-year post-OLT mortality, with CTP value of 10 and more and MELD scores of 25 and more having worse outcome at 1 and 5 years (65). These studies demonstrate that using MELD scores for liver allocation in this patient population may have distinct disadvantages, because patients undergoing retransplantation would need to be quite ill before reaching the top of the allocation list, thereby losing their "window of opportunity" to have a good outcome after retransplantation. Retransplantation at a lower MELD score would contribute to better outcomes. Other models have been proposed to determine which MELD score is associated with the greatest utility for retransplantation in patients with HCV and those without HCV, with the optimal outcome for retransplantation appearing to be achieved at MELD scores less than 28, with the maximal utility achieved at a MELD score of 21 for patients with HCV, and a MELD score of 24 for patients without HCV (57,71).

Living Donor Liver Transplantation and Retransplantation

With the increasing use of right lobe grafts for adult liver transplants, transplant surgeons and hepatologists will be faced with technical decisions regarding the use of living donors for retransplants, and ethical issues whether retransplantation should be performed in living donor recipients transplanted outside UNOS criteria, such as for large hepatocellular carcinomas. In a recent series of living donor liver transplants from the A2ALL consortium, 37 of 385 (9.6%) recipients of adult to adult living donor liver transplants required retransplantation, mostly for technical reasons (72). These technical issues markedly decreased as experience improved, and it may be that there will be less retransplantation in this group as technical complications decrease.

Summary

Along with the improvements in the results of primary grafting, a comparable trend is noted with the outcome of retransplantation. However, the national 1-year survival after retransplantation is currently approximately 65%, significantly less than primary OLT (2004 Annual Report SRTR). Certain clinical criteria have been found to affect the outcome of retransplantation. The most important factors appear to be the preoperative status of the recipient as indicated by ventilator dependence, renal failure, physical condition, age, and MELD score. The time interval to retransplantation, donor cold ischemia time, and donor quality also contribute to the overall outcome. The overall impact of retransplantation on the survival of all patients awaiting liver transplantation and the cost-effectiveness of this procedure are issues of current debates. What also remains controversial and unanswered is whether retransplantation for recurrent HCV has prohibitively poorer outcomes, as some analyses have identified the patient medical status as most predictive in this population, and it may be that improved judgment may also ensure better outcomes in this patient population (60,73).

Despite continued improvements, the generally inferior outcome has prompted many to question the

appropriateness of hepatic retransplantation on both economic and ethical grounds. On the other hand, an outright prohibition of hepatic retransplantation raises its own ethical questions of patient abandonment. In addition, limiting retransplantation would impede current efforts to expand the organ pool by utilization of marginal donors. The safety net of retransplantation is needed if an aggressive donor organ acceptance strategy is to be adopted by all transplant centers. Retransplantation is an essential treatment for patients undergoing liver transplant, who experience liver failure after their primary transplant. However, it must be applied with some discretion and careful decision making so that futility is avoided and maximal utility is achieved. Relying on MELD score alone to allocate organs seems insufficient in this patient population and needs to be studied. Futile transplants and retransplantation in subgroups of patients with little chance of successful outcome should be avoided. It is for this reason that mathematic models are being developed and tested in order to identify the subset of patients who will have acceptable outcomes and to identify factors that influence survival. These models and analyses should

provide valuable information on which sound clinical judgment for the selection of candidates most suitable and appropriate for retransplantation can be based.

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Chapter 60

Hepatobiliary Complications of Hematopoietic Cell Transplantation

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Key Concepts

- The most accurate prognostic indicator for the development of severe liver dysfunction is an early rise in liver function tests after hematopoietic cell transplantation (HCT).
- The most common causes of acute liver disease after HCT are acute graft versus host disease (GVHD), drug toxicity, and viral infection.
- Liver biopsy is often not necessary in classic presentations of GVHD and sinusoidal obstruction syndrome (SOS); however, the absence of full diagnostic criteria or the presence of other potential etiologies may indicate the need for histologic confirmation.
- Hepatitis B virus (HBV), hepatitis C virus (HCV), iron overload, and chronic GVHD are among the most common etiologies for chronic liver disease after HCT.
- The risk of HBV flare after HCT is highly dependent on the presence of detectable donor or recipient HBV deoxyribonucleic acid (DNA).
- The degree of hepatic iron overload present after HCT correlates well with the future development of hepatic fibrosis.
- Hepatic herpesvirus and fungal infections after HCT, although uncommon, can be life threatening and warrant immediate diagnosis and treatment.

Over the last few decades, hematopoietic cell (bone marrow or stem cell) transplantation (HCT) has evolved from an experimental procedure into a lifesaving, potentially curative therapy for patients with a wide variety of hematologic and oncologic diseases. It is now a recognized treatment option for refractory leukemia, lymphoma, thalassemia, aplastic anemia, sickle cell anemia, breast cancer, and immunodeficiency syndromes. Advances in preparative regimens, GVHD prophylaxis, and anti-infective prophylaxis and treatment have brought significant improvements in long-term disease-free survival after HCT.

Acute and chronic hepatobiliary complications remain the "Achilles' heel" of HCT and, despite advances in HCT, are responsible for significant morbidity and mortality after transplantation. Pretransplantation evaluation of donors and recipients and close monitoring after HCT reduces the development of severe hepatic complications. This chapter focuses on the specific common and

uncommon etiologies, diagnosis, and management of these complications.

Prevalence

The prevalence of chronic liver disease in HCT recipients varies in the literature, depending on sample size, length of follow-up, and method of diagnosis. Abnormal liver biochemistry test results in the first 12 months are common, particularly in recipients of allogeneic transplants (1). In this period, the most common causes of abnormal liver function in recipients of allogeneic transplants are GVHD, drug hepatotoxicity, and viral hepatitis. In autologous transplantations, disease recurrence and sepsis are predominant. Liver disease

may be responsible for up to 30% of deaths in HCT recipients (2). Recipients who have unrelated donors or donors with abnormal liver function have an increased risk of developing significant liver disease (2).

Chronic liver disease, often due to viral hepatitis, is seen in a high percentage of HCT recipients who survive long term (3). The largest study (>3,700 recipients) of long-term survivors showed a slow progression to cirrhosis (4). The cumulative incidences of cirrhosis 10 and 20 years after HCT were 0.6% and 3.8%, respectively. In comparison to matched controls without cirrhosis, only hepatitis C was found to be more prevalent in recipients.

Donor Evaluation

Once a suitable donor is selected, the evaluation should consist of liver function tests and serologic testing for infectious agents that are potentially transmissible to the immunosuppressed recipient (Table 60.1). Most notably, testing for previous exposure to hepatitis B virus (HBV), hepatitis C virus (HCV), and cytomegalovirus (CMV) provides an important estimate of the risk of transmission and the need for closer monitoring or prophylaxis. These etiologies are discussed in further detail in subsequent sections.

Recipient Diagnosis and Evaluation

The differential diagnosis of liver disease in this population depends on the specific period after HCT (Fig. 60.1). Liver function tests and clinical signs of liver disease (e.g., right upper quadrant pain, jaundice, ascites, weight gain) should be monitored frequently in the course of post-HCT care. Any suggestion of liver disease should be evaluated immediately as early diagnosis and treatment results in improved prognosis for most etiologies. The initial approach should be to evaluate for infection, review the recent drug history, and screen for viral hepatitis. Imaging tests may be helpful in all stages after HCT, particularly when sinusoidal obstruction syndrome (SOS), complications from gallstones, or the development of cirrhosis are suspected.

Table 60.1. Evaluation of Donor and Recipient in Hematopoietic Cell Transplantation

Donor	Recipient
Liver function tests	Liver function tests

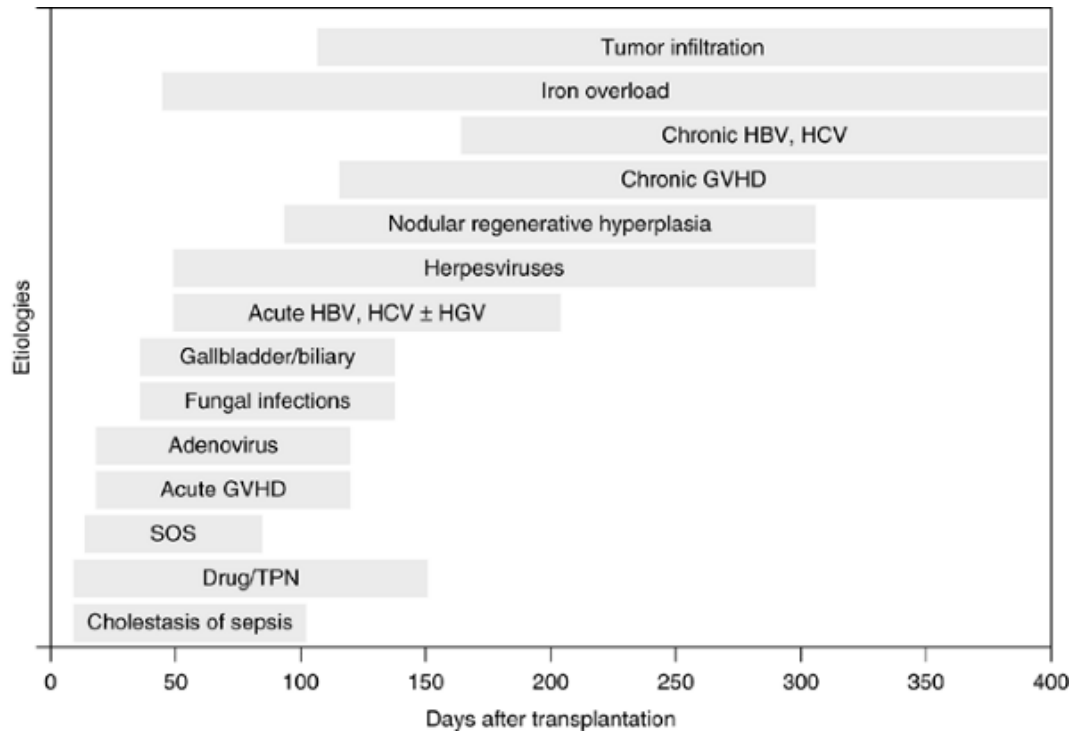
Hepatitis B virus	Hepatitis B virus
Surface antibody and antigen	Surface antibody and antigen
Core IgG	Core IgG
DNA	DNA
Hepatitis C antibody	Hepatitis C antibody
Cytomegalovirus IgG	Cytomegalovirus IgG
	Varicella zoster virus IgG
	Epstein-Barr virus IgG
	Iron studies
	Iron, TIBC, ferritin
	Hepatobiliary ultrasonography with vascular Doppler
IgG, immunoglobulin G; DNA, deoxyribonucleic acid; TIBC, total iron binding capacity.	

The use and safety of liver biopsy to evaluate abnormal liver function after HCT are debated. Clinical criteria for diagnosing specific etiologies, such as SOS and GVHD, may obviate the need for liver biopsy. However, a significant percentage of diagnoses and management decisions may be altered because of liver biopsy findings, particularly when risk factors for hepatitis B and C reactivation are present (5,6,7,8). Coagulopathy and thrombocytopenia can limit the ability to perform a safe liver biopsy. Percutaneous biopsy can be performed in patients with low bleeding risks (platelets >75,000 and international normalized ratio [INR] <1.5). Ultrasound-guided biopsy with a small cutting needle has been associated with a lower rate of complications in these patients (9). Transjugular biopsy with hepatic venous pressure gradient (HVPG) measurements may be safer and help identify patients with SOS in uncertain cases (5,7). A HVPG greater than 10 mm Hg highly correlates with the histologic diagnosis of SOS. In patients with platelet counts less than 50,000, laparoscopic liver biopsy may be indicated to control any potential bleeding complications (6).

Prognostic Indicators

An early rise in liver function test results after HCT may foreshadow a more severe clinical picture and worse prognosis. Within 200 days post-HCT, a bilirubin increase from 1 to 3 mg/dL is associated with a greater than sixfold increase in mortality (10,11).

Biopsy findings may be predictive of severe liver disease, often in direct relation to the number of characteristic findings and degree of necrosis on histopathology (12). Other factors associated with worse prognosis include advanced age, having an unrelated donor, and an abnormal alanine aminotransferase (ALT) level before HCT (13,14).



▪ **Figure 60.1** Causes of liver disease after hematopoietic cell transplantation. HBV, hepatitis B virus; HCV, hepatitis C virus; GVHD, graft versus host disease; HGV, hepatitis G virus; SOS, sinusoidal obstruction syndrome; TPN, total parenteral nutrition.

Common Complications

Graft Versus Host Disease

Acute graft versus host disease

Acute GVHD typically occurs between 15 and 100 days of HCT in up to 70% of allogeneic HCT recipients. T cells from the allogeneic graft recognize major and minor histocompatibility antigens on biliary cells, leading to lymphocytic intrahepatic biliary destruction. Rarely, an acute GVHD-like syndrome with identical histologic features can be seen in recipients of autologous transplants,

potentially caused by autologous T cells that react to self–major histocompatibility complex (MHC) class II antigens (15). Risk factors for the development of acute GVHD include age greater than 50, busulfan conditioning, and HCT recipients having unrelated or human leukocyte antigen (HLA)-mismatched donors (16,17,18,19). Organ involvement is typically less than that of chronic GVHD and is usually limited to the skin, gastrointestinal tract, and liver. Liver involvement often occurs after other organ involvement and is characterized by a cholestatic pattern of liver function tests.

Liver biopsy may not be necessary in a patient with skin or gastrointestinal GVHD who develops cholestatic parameters. However, if skin and gastrointestinal GVHD are not present or other etiologies, such as SOS, drug hepatotoxicity, or viral infection, are suspected, liver biopsy may be indicated. One study reported that suspected cases are classified as acute GVHD in only 43% of biopsies and that acute GVHD is picked up on biopsy in 17% of unsuspected cases; therefore, liver biopsy confirmation is almost always justifiable (7). Histologic findings of acute GVHD include lymphocytic involvement of intralobular bile ducts, bile duct epithelial cell dropout, and often a lack of significant inflammation because of early post-HCT immunosuppression. High levels of interleukin-6 and soluble interleukin-2 receptors are often seen early in patients who develop acute GVHD but may also be seen in SOS and sepsis (20,21). These tests have not been utilized clinically because of their expense and lack of specificity.

Prophylactic agents have had varied success in preventing acute GVHD after HCT. Immunosuppression with tacrolimus or cyclosporine and/or methotrexate is associated with a relatively lower incidence (19%

to 55%) of acute GVHD than those without prophylaxis, although toxicity from these agents may contribute significantly to morbidity (22,23,24,25,26,27). These studies have generally involved small numbers, are not placebo controlled, and have failed to show significant improvements in overall survival. Larger studies with cyclosporine prophylaxis have shown less acute GVHD, particularly when used in combination with methotrexate and corticosteroids (26,27). Ursodeoxycholic acid (12 mg/kg per day) given up to 90 days post-HCT significantly lowers the incidence of severe acute GVHD and is associated with an improved 1-year survival over placebo (28). Intravenous immunoglobulin is not effective in preventing GVHD over placebo (29).

Initial treatment of acute GVHD is usually with corticosteroids or cyclosporine. Both are more effective than antithymocyte globulin infusions and lead to resolution in approximately 40% of cases (26,30). Skin and gastrointestinal manifestations of GVHD respond better to corticosteroids than hepatic involvement (30). For steroid-refractory acute GVHD, options including mycophenolate mofetil, interleukin-2 receptor inhibitors (daclizumab), anti-CD54 antibodies (alemtuzumab), and rapamycin have shown some success in small studies (31,32,33,34). In severe, refractory cases, orthotopic liver transplantation (OLT) appears to be an acceptable treatment option for GVHD if other treatments fail. Data from reported cases and review of the national database of OLT for severe hepatic GVHD were examined recently (35). Post-OLT 1- and 5-year actuarial survivals were surprisingly high at 72.4% and 62.9%, respectively.

Chronic graft versus host disease

Chronic GVHD develops in 30% to 70% of patients after allogeneic HCT and is

more common in HCT recipients from unrelated donors (1,3,19). Other significant risk factors include age, busulfan conditioning, acute GVHD, varicella zoster virus (VZV) infection, and granulocyte colony-stimulating factor infusions (13,18,36,37). The distinction between acute and chronic GVHD is made on clinical and histologic grounds. Organ involvement tends to be more extensive than that in acute GVHD and, in addition to skin and gastrointestinal tract, may include exocrine glands, genitourinary tract, and lungs. MHC class II antigens are detected on the intrahepatic bile duct epithelium in patients with chronic GVHD and are involved in the signaling pathways leading to eventual destruction of these bile ducts (38). Portal involvement with lymphocytes and bile duct dropout, periportal fibrosis, piecemeal necrosis, and cholestasis are the predominant histologic features.

Liver test result abnormalities are typically cholestatic and slowly progressive in nature. Rises in alkaline phosphatase and 5′ nucleotidase levels are more specific for the diagnosis of chronic GVHD in comparison to elevation in bilirubin and aminotransferase levels (39). Patients with chronic GVHD can have a clinical and histologic presentation similar to that in patients with primary biliary cirrhosis (PBC) (40,41,42). Antimitochondrial antibodies may be seen in both, although autoantibodies are more likely to be false-positives in chronic GVHD. Biliary epithelial staining with pyruvate dehydrogenase complex-E2 specific monoclonal antibodies is present in the liver tissue of patients with PBC but not in those with chronic GVHD (41).

Acute hepatitis with aminotransferase levels greater than 1,000 U/L can be the first presenting sign of chronic GVHD, particularly in patients with recent reduction or cessation of immunosuppressant therapy (43,44,45,46). On liver biopsy, lobular hepatitis with hepatocyte necrosis and acidophil bodies are seen in comparison to the characteristic cholestatic features of chronic GVHD (44). Rapid reinstitution of immunosuppressive therapy results in outcomes and resolution similar to those in typical presentations of chronic GVHD. Ursodeoxycholic acid may be beneficial in these cases (45).

Prophylaxis for acute GVHD often leads to reduced rates of chronic GVHD. Tacrolimus and cyclosporine prophylaxis may reduce the incidence of chronic GVHD to 25% to 50% (24,27). Although most studies have not shown a survival benefit with prophylaxis, tacrolimus plus methotrexate has been associated with a 2-year disease-free survival of 50% in one study (23). Ursodeoxycholic acid does not reduce the incidence of chronic GVHD, but 1-year survival is improved mainly because of reduction in acute GVHD (28).

Initial treatment of chronic GVHD involves corticosteroids plus other immunomodulators. Refractory cases are seen in less than 30%. Salvage treatments result in response rates of 19% to 77% (31,32,47,48,49,50,51). Mycophenolate mofetil has the highest efficacy in refractory cases but may be associated with gastrointestinal side effects and opportunistic infections (31,47). Tacrolimus improves and normalizes liver test results in 60% and 33%, respectively (51). Lower response rates have been seen in cases treated with daclizumab (32). Ursodeoxycholic acid improves liver test results in the short term, but long-term treatment has not been evaluated (50). The success of OLT for refractory chronic GVHD was previously based on small case reports and series (52,53,54). When data from published studies and the national transplant database were combined, the 5-year survival after OLT for chronic GVHD was excellent at 62.9% (35). The rate of retransplantation was low and no patient developed recurrent GVHD. Therefore, OLT for either acute or chronic GVHD

appears to be a feasible option for severe, refractory cases.

Sinusoidal Obstruction Syndrome

SOS typically occurs within 30 days of HCT. Early studies showed a high incidence (54%) of mild to moderately severe SOS after HCT (55). Later reports with larger numbers showed that SOS occurred in a low percentage (4.7% to 5.3%) of recipients and that it was associated with a poor prognosis and death if clinically severe (56,57,58,59). Overall less than 20% die from SOS, whereas over 60% die from the severe form of the disease (59). Multiorgan failure, including fulminant hepatic failure, is associated with a near 100% fatality rate (56,57).

Risk factors for SOS in HCT are shown in Table 60.2. High-dose cytoreductive treatment is strongly associated with the development of SOS (55,59). Busulfan, cyclophosphamide and its metabolites, and combination treatment are the main offenders implicated (60). The risk of SOS is increased almost threefold with busulfan conditioning (57). Some have suggested monitoring the pharmacokinetics of busulfan by plotting area-under-curve (AUC) graphs because high AUC levels are associated with the development of SOS and subsequent mortality after HCT (61,62,63). The liposomal form of busulfan appears to have a lower incidence of SOS (64). Abnormal liver test results before HCT and HCV may also increase the risk of SOS (55,59,65,66,67). Other major pretransplantation risk factors include advanced disease status, high Karnofsky score, prior treatment with gemtuzumab ozogamicin, and allogeneic transplantation (11,59,68,69,70,71). Recently, $CD34^+$ selection in recipients of stem cell transplants was shown to lower the incidence of SOS (68,72).

Early histologic findings include central vein subendothelial thickening, sinusoidal dilatation, red blood cell extravasation outside the sinusoids, and pericentral hepatocyte necrosis. Local activation of the coagulation cascade and collagen deposition in the perivenular and subendothelial region results in reduction in blood flow from the sinusoids to the central veins (73). In addition, activated stellate cells, identified by immunohistochemical staining for α-smooth muscle actin antibodies, play an important role in the development of luminal narrowing and sinusoidal fibrosis (74,75). With ongoing obstruction and necrosis, the fibrosis can extend from these obliterated central veins. Terminal hepatic venule occlusion is not seen in all cases; therefore, the term *SOS*, rather than the old terminology veno-occlusive disease (VOD), more accurately reflects the pathophysiology (12). The number and degree of perivenular changes is associated with the clinical severity of SOS (12,76). These changes include hepatocyte necrosis, occluded venules, eccentric thickening of the venular subendothelium, phlebosclerosis, and perivenular fibrosis.

Table 60.2. Risk Factors for Sinusoidal Obstruction Syndrome in Hematopoietic Cell Transplantation

Definite	Suspected
High-dose conditioning	Glutathione-*S*-transferase M1 polymorphism

Busulfan	Immunoglobulin infusions
Cyclophosphamide	Unrelated or allogeneic transplant
Thiotepa	Norethisterone
Hepatitis C virus	Prior abdominal irradiation
Advanced disease status and Karnofsky score	
Abnormal liver enzymes	
Gemtuzumab ozogamicin	
T cell–depleted transplant	
Low protein C activity	

Activation of the coagulation cascade due to endothelial cell injury may precede the development of SOS. Levels of endogenous anticoagulants, protein C, and antithrombin III may be significantly reduced soon after HCT in patients who later develop moderate to severe SOS compared to those with mild or no SOS (77,78,79). Low levels of protein C are seen before and after cytoreductive therapy in those who develop SOS (80,81). Plasminogen activator inhibitor-1 (PAI-1) level is significantly elevated in SOS compared to other causes of liver disease after HCT (82,83,84). In addition, a significant decrease in PAI-1 levels with treatment of SOS is a good predictor of response (85). Others have shown that PAI-1 levels are not specific for SOS and are also elevated in sepsis, capillary leak syndrome, and GVHD (86). Procollagen type III level is also elevated in SOS and may be an early predictor for the development of SOS (87,88,89). Other factors seen with activation of the coagulation cascade and endothelial cell injury include d-dimer, tissue plasminogen activator (TPA), fibrinogen, vascular endothelial growth factor (VEGF), and hyaluronic acid (78,90,91,92). Although not typically used in clinical practice, knowledge of the levels, particularly of protein C, before and after HCT may indicate the need for reduced myeloablative therapy and closer monitoring for SOS.

Rapid diagnosis and intervention are critical in the management of SOS. Early intervention was associated with complete response in 76% and 50% of all and

severe cases of SOS, respectively (93). An increase in bilirubin level for 2 consecutive days within 20 days of HCT is significantly associated with the development of SOS (11). In clinical practice, the diagnosis of SOS is often made

on clinical grounds in the absence of alternative etiologies (55,66). Patients present with hepatomegaly, hyperbilirubinemia (>2 mg/dL), and weight gain (>5%) due to ascites formation. The diagnosis is secure in patients who meet these clinical criteria, although those with less than or equal to two criteria are less likely to have SOS and may need liver biopsy for confirmation (94). HVPG greater than 10 mm Hg correlates highly with the histologic diagnosis of SOS and may be useful when the biopsy or clinical scenario is inconclusive (3,7).

Noninvasive tests, such as Doppler ultrasonography and magnetic resonance imaging (MRI), may assist in making the diagnosis. Ultrasonographic findings, such as splenomegaly, ascites, low flow in the paraumbilical vein, and an elevated hepatic artery resistive index, correlate with the clinical and histologic diagnosis of SOS (95,96,97). Low portal vein velocity or portal vein thrombosis appear to be somewhat less reliable (79,98,99). Hepatic vein narrowing and gallbladder wall thickening may be seen on MRI in patients with SOS (100).

Before HCT, prophylactic strategies to prevent SOS are often employed. The most promising agents are ursodeoxycholic acid, heparin, and defibrotide (101). A pilot study and two of three randomized trials showed a significant reduction in the incidence of SOS with the use of ursodeoxycholic acid compared to placebo (28,102,103,104). Heparin, either unfractionated or low molecular weight, or in combination with defibrotide, is also effective in preventing SOS compared to placebo, with a low rate of bleeding complications (105,106,107,108,109,110).

Once SOS has developed, immediate attempts at treatment are required. Response rates depend on early diagnosis and severity of disease. An initial report on the compassionate use of defibrotide for severe SOS showed a response in 8 of 19 patients (111). Subsequent larger studies showed overall and severe disease response rates to be 55% to 76% and 36% to 50%, respectively (85,93,112). Defibrotide is preferred over other thrombolytic agents, such as recombinant TPA, that have a significantly higher rate of bleeding complications (113,114,115,116,117). Other less well-reported treatment strategies include antithrombin III, charcoal hemofiltration, *N*-acetylcysteine, and prostaglandin E_1 (118,119,120,121). For refractory cases, transjugular intrahepatic portosystemic shunt (TIPS) has been shown to reduce HVPG and improve abdominal pain and ascites but does not generally result in improved survival (122,123,124,125). In select cases, liver transplantation may be performed with a potential for long-term survival, although the data are sparse (53,126,127,128).

Hepatitis B Virus

Donor evaluation

HBV can be transmitted from donor to recipient and cause acute hepatitis soon after HCT. De novo HBV has been reported to occur in 3.2% of recipients (2). The risk of hepatitis relates to the presence of donor hepatitis B surface antigen (HBsAg) and replicating HBV deoxyribonucleic acid (DNA). (129) Hepatitis B carriers (HBsAg with little or no HBV DNA) less commonly transfer HBV to recipients, causing acute hepatitis (2,130). In recipients who convert from surface antibody positivity to negativity after HCT (reversed seroconversion), acute hepatitis does not occur if the donor is surface antigen negative but may occur if the donor is surface antibody negative (131,132).

Adoptive transfer of surface antibody from donor to recipient may result in loss of surface antigen and acquisition of surface antibody by the recipient

(131,133,134). In addition, donors who are vaccinated before donation may also transfer surface antibody to recipients (134,135). This may be particularly important in preventing acute flares of HBV after HCT and in long-term protection. Core antibody can also be transferred from donor to recipient and result in loss of surface antigen (136).

Risk of reactivation and acute flare

The most significant factor for developing severe HBV infection after HCT is the replicative status of the virus immediately before HCT, either in the donor or in the recipient. Recipients with surface antigen and undetectable HBV DNA have a significantly lower risk of reactivation than those with detectable DNA (130,137).

Precore or core promoter mutations in the viral genome predispose these patients to more severe reactivation and decompensation after HCT (130). The presence of recipient core antibody also poses a risk, particularly in the setting of detectable DNA and chronic GVHD (138,139). Finally, even patients with surface antibody who have presumed immunity can develop acute hepatitis after HCT, potentially related to low surface antibody titers and the presence of viral DNA in either blood or tissues (140,141). Fulminant hepatic failure can occur if the diagnosis is made late and therapy is not instituted in time (141,142).

Treatment

Acute reactivations of HBV, typically in the setting of immunosuppression tapering or withdrawal, are becoming less common with advances in the treatment and prophylaxis of HBV before and after HCT. Lamivudine is typically the first agent selected for HBV flares after HCT, unless the patient has a known lamivudine-resistant strain. In these patients, no data exist about

treatment with interferon and other agents associated with a low risk of viral resistance (e.g., adefovir, tenofovir, and entecavir). This being said, these agents are often used in patients with lamivudine-resistant HBV for lack of an alternative. Famciclovir, when added to lamivudine, may potentiate the antiviral effect (143). In addition, HBV replication has been shown to be suppressed by ganciclovir, which is given for CMV infection, although use of this drug primarily for HBV is not recommended (144). The length of treatment in all cases is also unknown. Those with reactivation during immunosuppression withdrawal should be treated until immunosuppressive agents are tapered or discontinued and for an undefined period thereafter.

Prevention

If possible, patients with chronic HBV should be treated before HCT with the goal of undetectable virus at the time of HCT. In this setting, treatment should be continued in patients long after engraftment and immunosuppression withdrawal. Patients who cannot wait until viral clearance with treatment should be given antiviral prophylaxis as soon as possible, at least 1 week before HCT. Recipients with surface antigen who are given lamivudine prophylaxis have a significantly higher 1-year survival (94% vs. 54%) and lower incidence of hepatitis flares (5% vs. 45%) and acute liver failure (0% vs. 15%) compared to those given no prophylaxis (145,146,147). A small study showed that famciclovir prophylaxis was associated with less reactivation and death than no prophylaxis (148). Another strategy involves vaccinating donors immediately before and/or after HCT. Seroconversion rates are surprisingly acceptable even in the period of

intense immunosuppression (149). This may prevent HBV flares once seroconversion takes place.

Hepatitis C Virus

The prevalence of HCV in HCT recipients is highly variable in the literature depending on patient number, year of publication, and indications for HCT. The largest study involving 63 transplantation centers reported that 5% of HCT patients were HCV antibody positive, although other studies have shown much higher percentages (31% to 51%), particularly in patients with thalassemia (150,151,152). Seventeen percent to 24% of patients are HCV ribonucleic acid (RNA) positive before HCT (67,151,153). HCV has been shown to be the etiologic factor in up to 50% of chronic liver disease after HCT (3). Most HCT recipients acquire HCV from previous blood transfusions, although donor-to-recipient transfer may occur in the setting of high donor HCV RNA levels (154). Transmission of HCV is currently low because of newer-generation HCV antibody testing and treatment of donors (87,155).

Risk of acute flare

Unlike HBV, patients rarely develop acute hepatitis or fulminant hepatic failure with withdrawal of immunosuppression after HCT (67,156,157,158). Although a common cause of chronic liver disease, HCV alone is not a predictor of the development of liver failure in patients undergoing HCT (159). However, HCV-positive recipients with abnormal liver enzyme levels before HCT have an increased risk of SOS-related liver failure and death (67,160). Therefore, patients with HCV should be monitored carefully for the development of SOS after HCT.

Treatment

Data about HCV treatment after HCT are sparse. One study involved 11 patients, 24 to 65 months post-HCT, who were treated with interferon for 6 to 12 months (161). Ten of 11 completed the protocol; 40% had a sustained virologic response and 50% had improved liver histology. Another smaller study of four HCT patients showed a 50% end-of-treatment response rate at 12 months with ribavirin monotherapy (162).

Long-term prognosis

Progressive fibrosis and clinical deterioration from HCV has been reported in multiple studies in HCT recipients (153,163,164,165). The median time to the development of cirrhosis is significantly shorter in HCT recipients—18 years compared to 40 years in non-HCT controls (166). Other studies have shown that HCV does not lead to higher morbidity and mortality long term after HCT (67,167). What might differentiate those with mild liver disease from those with progressive fibrosis is the hepatic iron load, particularly in patients undergoing transplantation for thalassemia. Patients with HCV and heavy iron deposition have a significantly higher incidence of hepatic fibrosis than those without iron deposition (168). Other potential risk factors for progression are HCV genotype and the presence of extrahepatic manifestations of HCV (166,169).

Iron Overload

Iron overload may be responsible for a significant percentage of chronic liver disease in patients after HCT (3,170). Hepatic iron content increases shortly after

HCT and is associated with an elevated ferritin level (171,172). However, progressive fibrosis due to hepatic iron deposition in HCT recipients is most commonly seen in patients with a pre-HCT history of iron overload from multiple blood transfusions (thalassemia) (173). Studies have also shown that the presence of severe iron overload significantly impacts survival and transplantation-related mortality (174,175). Those

who have hepatomegaly and fibrosis before HCT will invariably develop progressive iron overload and fibrosis years after HCT (173). This degree of progression correlates well with the hepatic iron content and the presence of HCV (168).

The diagnosis of iron overload is often made by an elevated ferritin and transferrin saturation. Liver biopsy may not be necessary in the right clinical context, although hepatic iron stores often reflect total body iron stores accurately and may give prognostic information (176). Iron overload suggestion on MRI examination is common after HCT and may correlate with the number of previous blood transfusions (177).

Initial detection of significant iron overload may identify those patients at highest risk for adverse post-HCT outcomes. In small studies, early iron reduction with phelobotomy or iron chelation therapy has been shown to be safe and effective in maintaining low ferritin levels immediately after HCT (178,179,180). Iron reduction is, however, better tolerated after HCT when adequate return of red cell production by the bone marrow occurs. Studies have shown significant improvements in ferritin level, transferrin saturation, liver enzyme levels, hepatic iron concentration, and fibrosis scores in patients with undergoing phlebotomy years after HCT (5,176,181).

Common Causes of Cholestasis

Sepsis

Sepsis of any cause, particularly in the setting of neutropenia or high levels of immunosuppression, may lead to biochemical liver abnormalities and cholestasis (182). The pathophysiologic features of cholestasis of sepsis are similar in the general and transplantation populations. Canalicular cholestasis is the most common histologic finding, typically with dilated periportal ductules and occasionally inspissated bile in cholangioles. Other nonspecific findings include steatosis and pericentral ischemic necrosis, potentially related to poor perfusion from sepsis (183). In severe septic shock, jaundice may be marked. Laboratory studies have shown that cytokine release in the circulation related to bacterial lipopolysaccharides downregulates bile acid transport and bile secretion (184). Ductal obstruction resulting in jaundice can be easily differentiated from cholestasis of sepsis by ultrasonography and histologic features of nonsuppurative cholestatic injury (185). Resolution of sepsis invariably results in normalization of bilirubin and cholestasis.

Total parenteral nutrition

Determining that total parenteral nutrition (TPN) is the primary cause of cholestasis after HCT is challenging. Multiple other factors are often present, such as sepsis, drugs, viral infections, and GVHD. Cholestasis related to TPN is much more common and severe in infants compared to older children and adults. Typically in adults, increases in levels of serum transaminases, alkaline

phosphatase, and γ-glutamyl transpeptidase (GGT) are seen within 3 weeks of TPN initiation and return to normal in over 75% of patients (186,187). The most common histologic abnormalities include intrahepatic cholestasis, portal/periportal fibrosis, and periportal macrovesicular steatosis (188). This fibrosis may persist but does not generally progress after TPN discontinuation. Progression to cirrhosis and the development of clinically significant liver disease is uncommon in adults receiving long-term TPN. The initial management of TPN cholestasis involves reduction of dextrose content in TPN with conversion to more lipid-based calories and cycling infusions to allow "TPN-free" periods to improve biliary flow. Progressive cholestasis, despite these measures, should warrant discontinuation of TPN if possible.

Drugs

Numerous medications given after HCT can lead to intrahepatic cholestasis and liver test abnormalities. Hepatotoxic agents cause either dose-dependent intrinsic liver injury or dose-independent idiosyncratic toxicity, often related to an immunologic or metabolic reaction. Many chemotherapeutic agents and corticosteroids can cause steatosis, SOS, cholestasis, and/or hepatocellular injury. Prophylactic or therapeutic anti-infective agents such as sulfonamides, macrolides, cephalosporins, penicillins, and azole antifungals can lead to significant cholestasis, vanishing bile duct syndrome, or phospholipidosis. High doses of nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac, diclofenac, and indomethacin, may cause cholestasis and/or hepatocellular injury. Lastly, agents used for GVHD, for example, cyclosporine and less commonly tacrolimus, can lead to cholestasis in the setting of high serum and tissue levels of these agents (189). Histologic changes consist of bile duct epithelium hypertrophy with cytoplasmic vacuoles and foamy deposition within the hepatic sinusoids. In rat models, cyclosporine decreases bile flow and bile salt secretion (190).

Uncommon Complications

Herpesvirus Infections

Although herpesvirus infections are well described in HCT recipients, the literature, specifically on hepatic involvement, is sparse. Whether this represents a lack of reporting or a true low incidence is unknown. Advances in antiviral prophylaxis and early recognition

and treatment are major factors lowering the risk of viral dissemination.

Herpes simplex virus 1 and 2

Fulminant hepatitis due to herpes simplex virus (HSV) early after HCT has been reported, although incidence data specific for herpes hepatitis in this population are not available (191). Risk factors for reactivation include pretransplantation conditioning, HSV seropositivity, and a history of leukemia (192). Herpes hepatitis typically causes massive coagulative necrosis with purple nuclear inclusions surrounding the regions of necrosis. Skin lesions are not always present, making the diagnosis more difficult. HSV DNA detected in peripheral leukocytes is more specific for disseminated infection (e.g., esophagitis, hepatitis) and may have predictive value when its level is rapidly rising (193,194).

Immediate treatment with intravenous acyclovir is warranted. Prophylactic strategies after HCT, including use of acyclovir and its derivatives, have been shown to lower the incidence of HSV infections in general. However, the effect of prophylaxis on viral dissemination, including hepatitis, has not been reported probably because of the low incidence of this complication (195,196,197). Acyclovir- and foscarnet-resistant HSV strains have been increasing with use of prophylaxis, although they have not been associated with a higher risk of dissemination (195,198,199).

Varicella zoster virus

The incidence of VZV infections has been declining, likely because of antiviral prophylaxis and less intense immunosuppression (200,201,202). Although the overall incidence of disseminated VZV after transplantation has been reported to be approximately 5%, those who develop skin lesions (chicken pox) have a greater chance (11% to 33%) of subsequently developing organ involvement. (200,203,204). Infections typically occur within the first year of transplantation and often occur in the setting of GVHD, pretransplantation VZV seropositivity, and recent discontinuation of antiviral prophylaxis (37,200,201,203,204,205). Similar to HSV, VZV dissemination without skin involvement can occur in the presence of high VZV DNA levels (206,207). Clinically, patients with VZV hepatitis may present with severe abdominal pain and significant liver enzyme level elevation (208). Fulminant hepatic failure from varicella hepatitis, although uncommon, has been reported after HCT (209). Hepatic lesions are similar to HSV hepatitis, with necrosis and a paucity of significant inflammation. Electron microscopy, immunocytochemistry, and polymerase chain reaction (PCR) analysis may help differentiate VZV from other herpesviruses (183).

Morbidity after VZV infections is high. However, with prompt antiviral treatment, death from dissemination is rare (200,203,204,205). Acyclovir is the drug of choice for treatment (210). For prophylaxis, acyclovir reduces VZV reactivation significantly, although VZV can occur soon after prophylaxis is stopped. Longer-term prophylaxis may be considered in patients at high risk for VZV (i.e., previous zoster, GVHD) (201). Varicella vaccination given before and after HCT has been shown to significantly reduce zoster infections and the potential for viral dissemination (211,212).

Epstein-Barr virus

Infection with Epstein-Barr virus (EBV) after HCT rarely involves the liver alone. Proliferation of EBV in the setting of high immunosuppression, antilymphocyte therapy, and T-cell depletion results in B-cell proliferation and the potential for post-transplantation lymphoproliferative disorder (PTLD) (213,214,215,216). Critical to controlling EBV proliferation is the presence of $CD8^+$-specific T cells (217,218). Clinical signs, such as fever, lymphadenopathy, splenomegaly, weight loss, abnormal aminotransferase levels, and bone marrow suppression, are common. Histopathology shows a diffuse lymphocytic infiltrate in the sinusoids and occasionally focal apoptotic cells (219). Rapid increases in serum EBV DNA levels greater than 10^4 is highly associated with the development of PTLD after HCT, suggesting the need for monitoring viral load (220,221,222,223). In addition, rapid reduction in DNA levels with treatment predicts a successful response (224).

Studies have shown that preemptive treatment with an anti-CD20 antibody, rituximab, when EBV DNA levels greater than 10^3 or $CD8^+$ responses to EBV are

detected, may prevent the subsequent development of PTLD (218,225,226). Infusion of EBV-specific cytotoxic T cells at the time of HCT or at peak EBV viral load may reduce viremia and the risk of PTLD (227,228). Polyclonal EBV infections typically respond to reduction in immunosuppression, while various treatments for PTLD have had varied success, including rituximab, standard lymphoma chemotherapy, ganciclovir, and α-interferon (229,230).

Cytomegalovirus

Little data exist on the incidence of CMV hepatitis after HCT. A higher rate of CMV infection is seen in recipients of CD34-selected peripheral blood stem cell transplants, alemtuzumab infusions, and transfusions without leukocyte depletion or CMV screening (231,232,233,234,235). Patients may present with a variety of signs and symptoms, including fever, cytopenia, colitis, pneumonitis, and abnormal transaminases. The pathognomonic findings are an enlarged hepatocyte, bile duct epithelium or endothelial cell containing

cytoplasmic basophilic granules, and an intranuclear inclusion surrounded by a clear halo (“owl's eye”). Blood CMV DNA testing is more sensitive than culture or antigen testing and may predict the development of CMV end-organ disease.

Antiviral prophylaxis after HCT, including acyclovir, valacyclovir, ganciclovir, and valganciclovir, are all highly successful in reducing the incidence of CMV infection (236,237,238,239,240). Intravenous ganciclovir is the treatment of choice for known infections, with foscarnet given for known ganciclovir-resistant strains. Another approach (preemptive) is to closely monitor for CMV infection by culture, antigen, or DNA and only treat once CMV is detected (241,242,243). Compared to culture, monitoring CMV DNA frequently after HCT and treating once DNA is detected has been found to reduce the subsequent development of symptomatic CMV disease and related mortality (244,245,246). Isolation and donor transfer of T cells with specific activity against CMV target antigens may reconstitute cellular immunity against CMV and may represent a novel approach for future treatment (247,248).

Human herpesvirus-6

Infection with human herpesvirus-6 (HHV-6) in transplant recipients may result in a lobular, nonspecific hepatitis (183,249). This virus can be transmitted from donor to recipient, resulting in an early post-transplantation hepatitis (250). The detection of HHV6 early after HCT may be associated with delayed engraftment (251). A high percentage of HCT recipients have HHV6 viremia, but symptoms and disease specifically related to HHV6 are uncommon (252). Bone marrow suppression and gastrointestinal involvement are the most common presenting signs. Cidofovir and foscarnet appear to have the highest antiviral activity against HHV6 (253).

Adenovirus

Adenovirus infection after HCT is rare but may lead to acute hepatic failure in the setting of induction chemotherapy, high levels of immunosuppression, and GVHD (254,255,256,257,258). Overall, adenovirus infection is seen in up to 5% of recipients and presents in a manner similar to that of CMV, with pneumonia, gastroenteritis, hepatitis, and less commonly encephalitis and hemorrhagic cystitis (257,258). Fulminant hepatic necrosis with intranuclear inclusions is seen. Differentiating adenovirus from HSV hepatitis can be difficult and is aided

by electron microscopy and immunohistochemical staining (256). Treatment with antiviral agents, such as cidofovir and ribavirin, has had limited success (255,259).

Hepatitis G Virus

Hepatitis G virus (HGV) is a transfusion-transmitted flavivirus often seen in HCT recipients with HCV. A significant percentage (15% to 48%) of transplant recipients have detectable HGV RNA levels before and after HCT (153,260,261,262,263). The only real consequence of HGV infection may be delayed transplant engraftment (264). Although higher transaminase levels may be seen in HCT recipients with HGV, clinically significant hepatitis or chronic liver disease is not thought to be caused by HGV alone (261,263,265). The hepatic complications of HGV infection may only be a result of HCV coinfection (153,260). No specific treatment is recommended in HGV-infected HCT recipients. While treatment with interferon for HCV may clear HGV in immunocompetent patients, it is not known whether this occurs in immunocompromised HCT recipients (266,267). It is also not know whether HCV is more difficult to treat in HCT patients with HGV.

Fungal Infections

In the early days of HCT, up to 9% of cases were found on autopsy to have fungal involvement of the liver (268). Currently, this entity, particularly hepatosplenic candidiasis, is uncommon after HCT and more commonly seen in patients before HCT. Risk factors for fungal liver involvement after HCT include superficial infections, deep fungal infections, and severe liver dysfunction from SOS or GVHD (268). The most common fungal infections involving the liver are candidiasis, aspergillosis, and zygomycosis, but other mycoses, such as cryptococcosis, histoplasmosis, blastomycosis, and coccidiomycosis, can be seen depending on the regional prevalence. Hepatosplenic candidiasis can present with multiple portal and periportal abscesses and granulomas, with yeast and hyphae present in the sinusoids (183). Before HCT, patients can be treated aggressively with amphotericin to eradicate candidiasis and still successfully undergo HCT (269,270). Aspergilli (*Aspergillus fumigates* and *Aspergillus flavus*) can cause hemorrhagic hepatic necrosis and local infarction because of blood vessel invasion (183). Zygomycosis is an infection caused by a group of fungal organisms, such as *Rhizopus*, *Mucor*, and *Absidia*, which can proliferate in the setting of immunosuppression and transplantation. Similar to aspergillosis, widespread vascular invasion from zygomycosis causes ischemic necrosis and multiple, necrotic hepatic nodules.

Assessing the size, morphology, and special staining on histology typically confirms the diagnosis of fungus in the liver. Culture of liver tissue and blood, although often of low yield, is the most specific test. Others have proposed using panfungal PCR assays to allow for a rapid early diagnosis (271). Imaging tests such as ultrasonography and computed tomography (CT)

scan, particularly for hepatosplenic candidiasis, may provide additional evidence for invasive hepatic infections, although the sensitivity is quite low (268,272). Fungal abscesses can only be seen on imaging tests after neutropenia resolves post-transplantation. The treatment of invasive hepatic infections depends on the organism and includes the use of amphotericin B, azole antifungals, and echinocandins. Post-HCT prophylaxis with itraconazole, fluconazole, or low-dose

amphotericin can significantly reduce the incidence, morbidity, and mortality associated with invasive fungal infections (273,274,275).

Biliary Complications

Gallbladder disorders

While gallbladder sludge and cholelithiasis are commonly seen in HCT recipients, complications such as cholecystitis, gallstone ileus, and choledocholithiasis are uncommon (98,276). Gallbladder sludge after HCT contains nonspecific residue, calcium-binding protein, and calcium bilirubinate crystals (277). The formation of sludge occurs early after HCT and often progresses to stone formation, unrelated to the conditioning regimen or gallbladder contractility (278). Cholecystitis can occur after HCT in a small percentage of patients with gallstones and is treated with either cholecystectomy or nonsurgical measures (e.g., antibiotics, percutaneous drainage) in poor surgical candidates (279,280). Acalculous cholecystitis is even less common and may be associated with prolonged fasting, SOS, transfusions, and the use of TPN (280,281). If possible, the best chance for survival is cholecystectomy as acalculous cholecystitis is associated with gallbladder wall necrosis and perforation.

Biliary obstruction

New hepatobiliary abnormalities are common in the first few months after HCT and are often multifactorial (98). Diagnosing biliary obstruction as a cause of jaundice may be difficult when other disorders are present and bile ducts are not particularly dilated on imaging. The incidence of biliary obstruction after HCT was reported to be 0.1% in one large study involving two major transplantation centers (282). Of the small number of cases reported, the etiologies were biliary sludge, choledocholithiasis, duodenal hematoma, stricture, recurrent malignancy, and EBV-associated PTLD (282). Chronic GVHD and peribiliary chloroma may also cause biliary obstruction (283,284). An experimental model of GVHD in mice has shown that the early nonsuppurative lymphocytic cholangitis may evolve into ductal fibrosis of intra- and extrahepatic bile ducts, similar to that seen in primary sclerosing cholangitis (285).

Tumor Infiltration

Recurrent and secondary malignancies can involve the liver after HCT. Leukemia and lymphoma may infiltrate the liver, leading to hepatomegaly and high levels of alkaline phosphatase (286,287,288). Solid cancers occur almost twice as frequently in HCT recipients than the healthy population (289). Hepatocellular carcinoma may occur at a higher rate after HCT in patients with HBV, HCV, or cirrhosis of any cause (289). Bile duct adenocarcinoma leading to obstructive jaundice after HCT has also been reported (290). Biopsy is important in differentiating malignancy from infections and regenerative nodules (291).

Nodular Regenerative Hyperplasia

Nodular regenerative hyperplasia is characterized by idiopathic, non-neoplastic nodule formation in the absence of hepatic cirrhosis. Most nodules are less than 1.0 cm in size and distort the hepatic architecture enough to cause portal hypertension. The diagnosis after HCT is often confused with that of SOS and can sometimes only be determined on laparotomy or autopsy (292,293). Ascites is the most common portal hypertensive complication. The etiology is unknown and

is not thought to be related to age, the underlying disease, or cytoreductive regimens (292). The treatment is directed at the symptoms related to portal hypertension. Nodular regenerative hyperplasia may resolve spontaneously months after HCT and typically does not contribute to significant morbidity or mortality (292).

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